THE ANALYST

The Documentation of Molecular Spectra

THE Infra-red Absorption Data Joint Committee, which was set up in 1951 by the Chemical Society to advise on the publication of spectral data, made in 1952 an interim report with recommendations designed to raise the quality of infra-red absorption spectra published in scientific journals. These recommendations have since been accepted in principle by most British societies, and the relevant parts were adopted by the Publication Committee so that the format of published spectra should be standardised (see *Analyst*, 1953, **78**, 684).

The recommendations, however, placed considerable limitations on the publication of infra-red spectra, and in its report the Committee drew attention to a consequential need, namely, the preparation in some form of a register or card index of the spectra of pure compounds; the Committee has since studied various British, American and European schemes for cataloguing and indexing spectra. A system of Keysort punched cards, in which the holes around the edges are coded and slotted according to the spectral and structural characteristics of the compound and in which the cards can be sorted by manual methods, appeared to offer the best solution. The Committee then learnt of a somewhat similar scheme that was being developed by the Gesellschaft für Spektrochemie und angewandte Spektroskopie in Germany. It became clear that a union of the German and British schemes would be mutually beneficial and might have advantages leading to a collection of universal value. A joint plan has now been agreed upon and the first cards are about to be distributed (see H. W. Thompson, *J. Chem. Soc.*, 1955, 4501).

Two identical editions of Keysort cards will be published in English and German, the publishers being Butterworths Scientific Publications, London, and Verlag Chemie, Weinheim, respectively. Initially, the cards will deal only with infra-red and Raman spectra, but it is hoped to extend the scheme later to ultra-violet spectra.

Two separate cards, namely, the spectral and literature cards, will be issued in each edition. The molecular and structural formulae, the main physical properties and the spectrum of the compound are printed on the spectral cards; literature references that may relate either to spectra described on the spectral cards or to important developments in either technique or theory are recorded on the literature cards. The 203 holes around the edges of the spectral card are coded for the structural features of the compound and for its principal absorption bands; those around the edges of the literature card are used to record names of authors, year of publication, an indication of the nature of the work (*e.g.*, analysis) the spectral range studied, the state of aggregation of the sample and details of either the equipment, method or theoretical aspects discussed in the paper.

It is to be hoped that this Anglo-German venture will provide a satisfactory basis for a wide international scheme of cataloguing spectral data.

J. E. PAGE

A Visit to Cambridge

A FEW weeks ago some of us spent a very enjoyable morning revisiting favourite places at Cambridge, such as Trinity Hall, King's College Chapel, the Backs, the old-fashioned market and Parker's Piece, where, it is understood, Jack Hobbs first played the game.

We then went along to meet a very distinguished crowd of visitors to Cambridge, including Professors of Physical Chemistry, industrial users of the polarograph, Government representatives and lecturers from Universities and Technical Colleges. After this reception we were fortunate to hear a very interesting talk by the Research Director of a particular firm that makes polarographic equipment. This was concerned in the main with the development of the polarograph; one of the original polarographs was on view. We like the story the Research Director told of how, in the early days of the development, a foreign visitor was working there. When he saw the step due to a particular ion being traced and re-traced on the polarograph, he had only one expression for it, this being "what nice."

A new Polarographic Research Laboratory was then opened by Professor J. Heyrovský, who made no secret of the fact that for him "all is polarography."

A tour was made of the Polarographic Research Laboratory and the General Research Laboratories of the company and this was followed by a visit to the University Research Laboratories.

Travelling home after a full day we felt that our time had been well spent. It seemed to us that an industrial company making chemicals takes it for granted that technical service about its products should be provided for the customer. Here was a company of instrument manufacturers setting up a laboratory to give just such service, *i.e.*, to carry out research on improvements of the instrument and, more important for the analyst, to indicate how the polarograph can be used to carry out particular tests. How often have we gone along to instrument manufacturers who can prove to us that they have manufactured a first-class instrument but who seem relatively unconcerned about its use. In short, it seems to us to be a good thing that trained analysts should be finding their way into firms making polarographs and other equipment; it must be noted that other polarographic firms in this country are showing equal keenness.

There were other questions that interested us; for example, what is to be the real function of the polarographs finding their way on to the market, *i.e.*, such instruments as the cathode-ray polarograph, the square-wave polarograph and the polarograph with the Univector attachment. Are we only to gain in sensitivity and specificity by their use, or is the time coming when the polarograph will be so accurate an instrument that some of our traditional volumetric and gravimetric procedures will have to take second place to the polarographic method?

J. HASLAM

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETING

An Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, April 4th, 1956, in the meeting room of the Chemical Society, Burlington House, London, W.1. In the absence of the President, the Chair was taken by Dr. D. W. Kent-Jones, F.R.I.C.

The following papers were presented and discussed: "The Determination of 4-Chloro-2-methylphenoxyacetic Acid in MCPA by a Differential Refractometric Method," by R. Hill, B.Sc., A.R.I.C.; "Paper Chromatography with Continuous Change in Solvent Composition. Part I: Separation of Fatty Acids. Part II: Separation of Surface-active Agents," by F. Franks, B.Sc., A.R.I.C.

NEW MEMBERS

Ordinary Members

Aubrey Edward Alldridge, B.Sc. (Nottingham); William Jeffery Beer; Kai-Chih Chou, B.Sc.; Stuart Collings, A.R.I.C.; William Dunnet, A.R.I.C., A.H.-W.C.; Thomas Talbot Gorsuch, B.Sc. (Lond.), A.R.I.C.; Ernest Guy, B.Sc. (Lond.); Frederick Wesley Hares, A.R.I.C.; Brian Percival Harold James, B.Sc. (Southampton), A.R.I.C.; Anthony Harold Latimer, B.Sc. (Lond.); Robert Donald MacDonald, B.Sc. (Lond.); William James Murray; Michael William Robertson, B.Sc. (Lond.), A.R.I.C.; Kenneth Charles Sellers, B.Sc. (Lond.), Ph.D. (Cantab.), M.R.C.V.S., D.V.S.M.; Jack Stanley Wright.

Junior Member

Donald Joseph Coplin, B.Sc. (Nottingham).

DEATH

We record with regret the death of

David Agnew Griffith.

NORTH OF ENGLAND SECTION

An Ordinary Meeting of the Section was held at 2.15 p.m. on Saturday, March 24th, 1956, at the City Laboratories, Mount Pleasant, Liverpool. The Chair was taken by the Chairman of the Section, Mr. J. R. Walmsley, A.M.C.T., F.R.I.C., F.P.S.

The following paper was presented and discussed: "New Reagents and New Developments in the Fine Chemical Field," by W. C. Johnson, M.B.E., F.R.I.C.

SCOTTISH SECTION

An Ordinary Meeting of the Section was held at 7.15 p.m. on Thursday, March 22nd, 1956, at the Central Station Hotel, Glasgow. The Chair was taken by the Chairman of the Section, Dr. F. J. Elliott, F.R.I.C., F.R.S.E.

The following papers were presented and discussed: "The Determination of Calcium in Plant Material by Flame Photometry," by R. G. Hemingway, M.Sc.; "The Flame Photometer in Silicate Analysis," by A. J. Shorter, M.Sc., M.S., M.Inst.F., A.R.I.C.; "A New System of Reporting and Recording Analytical Results," by A. O. Pearson, B.Sc., F.R.I.C.

MIDLANDS SECTION

An Ordinary Meeting of the Section was held at 7 p.m. on Tuesday, March 6th, 1956, in the Main Chemistry Theatre, The University, Edgbaston, Birmingham 15. The Chair was taken by the Chairman of the Section, Mr. J. R. Leech, J.P.

A lecture was given on "Modern Qualitative Analysis and Industrial Practice" by Professor Dr. C. J. van Nieuwenburg.

MICROCHEMISTRY GROUP

The fourth London Discussion Meeting of the Group was held on Wednesday, February 22nd, 1956, at 6.30 p.m., in "The Feathers," Tudor Street, London, E.C.4. In the absence of the Chairman of the Group, the Chair was taken by the Honorary Secretary, Mr. D. W. Wilson, M.Sc., F.R.I.C.

Dr. R. Belcher, F.R.I.C., F.Inst.F., and Mr. H. Holness, M.Sc., F.R.I.C., introduced the subject of "Small-scale Qualitative Inorganic Analysis," after which there was an informal discussion.

PHYSICAL METHODS GROUP

THE 52nd Ordinary Meeting of the Group was held at 6.30 p.m. on Tuesday, February 14th, 1956, in the meeting room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the Chairman of the Group, Dr. J. E. Page, F.R.I.C.

The subject of the meeting was "Polarography" and the following papers were presented and discussed: "A Comparison of Three Highly Sensitive Polarographs," by D. J. Ferrett, D.Phil., G. W. C. Milner, M.Sc., F.R.I.C., H. I. Shalgosky, B.Sc., A.R.I.C., and L. J. Slee, B.Sc.; "Polarography of the Dithionite (Hydrosulphite) Anion and Some Related Oxyacids of Sulphur," by W. Furness, B.Sc., Ph.D., F.R.I.C.; "The Polarographic Determination of Uranium in Ores," by H. I. Shalgosky, B.Sc., A.R.I.C.

Obituary

HORATIO BALLANTYNE

HORATIO BALLANTYNE, whose fame was as an expert witness and who was acknowledged as one of the greatest chemical authorities on patents, died at his home near Tadworth, Surrey, on January 25th, at the age of 84. His reputation as a chemist in the Courts is so well known that it seems unnecessary to emphasise this. There were few important cases, patent or otherwise, in which he did not appear. Quiet, unassuming and cool, he was a model for all expert witnesses. It was therefore not surprising that *The Times* of January 27th reported a moving appreciation of him by that great patent lawyer, Mr. Justice Lloyd-Jacob.

Born in Glasgow in 1871, Ballantyne's early training as a chemist was in the laboratory of the Glasgow City Analyst. He had further experience with Wallace, Tatlock and Clark and later with Tatlock, Thomson and Redman. It was in 1896 that Ballantyne, still a young man, started his practice in London, and he soon acquired a deservedly great reputation. Most of us only remember him from the early years of the century and particularly after the first World War, when he was at the height of his fame and greatly in demand, since he had a lawyer's appreciation and understanding of patents and patent law, in addition to his exceptionally wide chemical knowledge and experience. Indeed it was partly the width of his knowledge that made him so able and so excellent a witness; fortunately he also had the ability to expound his views clearly and convincingly.

It is well known that he gave up his practice in 1928 to join the Board of Unilever Ltd., a Company for whom he had acted and whom he advised on many, almost historic, occasions in court. Ballantyne was a member of many Government Committees; he will be expecially remembered in this phase of his career as a member of the Inter-Departmental Committee dealing with awards for inventions by Government servants and as a member of the Board of Trade Committee on Patent Law and Practice in 1929.

What may not always be remembered was that Ballantyne was a keen supporter of the Society and that he was a member of Council three times, namely in 1903–04, 1910–11 and 1933–34. He appreciated to the full the value of the information given by proper chemical analysis. Undoubtedly his early training in the laboratories of public analysts had made a great impression on his mind. It was a privilege to see him as he went round the laboratory of a firm, probably in connection with a pending patent action, quietly and unobtrusively, but never failing to grasp the essentials of the problem in hand.

He was a Fellow of the Royal Institute of Chemistry and served as a member of Council twice, 1899–1901 and 1915–1918, and was also twice a Vice-President, 1918–1919 and 1920–23.

Naturally the Society had not seen much of him in recent years and indeed he only occasionally visited Unilever House, but he still kept in close touch with chemical literature Many of us are proud simply to recall the privilege we had in working with him on many occasions.

D. W. KENT-JONES

The Development of Polarographic Analysis

By J. HEYROVSKÝ

(Shortened version of the lecture delivered at the meeting of the Society on Tuesday, November 29th, 1955)

ALTHOUGH the very first polarograms[1] obtained 30 years ago neatly showed the "waves" or diffusion currents due to traces of metals, from which the use of such curves for qualitative and quantative analysis at once became evident, this discovery was not widely known until after 1936. In that year the noted German analyst W. Böttger, Professor at Leipzig, published in his textbook "*Physikalische Methoden der analytischen Chemie*" the author's account of the polarographic method. By this publication the polarographic method was acknowledged as an analytical procedure and its description has since been incorporated into analytical textbooks.

The meaning of the word "polarography" needs to be explained. According to the author's view, polarography is the science of studying the processes occurring around the dropping-mercury electrode. It includes not only the study of current - voltage curves, but also of other relationships, such as the current - time curves for single drops, potential - time

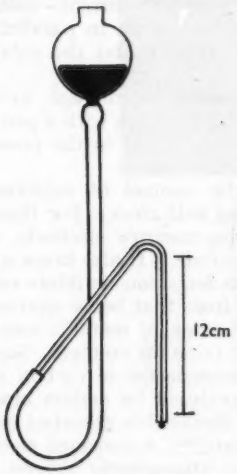

Fig. 1. Mašek's bent capillary

Fig. 2. Smolef's horizontal capillary

curves, electrocapillary phenomena and the streaming of electrolytes, and its tools include, besides the polarograph, the microscope, the string galvanometer and even the cathode-ray oscillograph.

The reason why so much study is concentrated upon the small mercury drop is its unique property of giving exactly reproducible results, *i.e.*, because the mean current passing through the solution is dependent only on the applied potential and is independent of time and of the direction of the polarising voltage. No other electrode has this property, except the streaming-mercury (mercury-jet) electrode. Solid electrodes, *e.g.*, rotating or vibrating platinum electrodes, give a curve that changes when the applied potential is reversed or when the rate at which the voltage increases is varied. As the results are not reproducible, there is no sound experimental basis for a theory; in fact, there are no theoretical considerations relating to the electrode processes at solid electrodes. The term "polarography" is therefore restricted to the mercury capillary electrodes.

As regards the shape of capillaries, long capillaries, if connected to the reservoir by flexible tubing, should be bent to the form (Fig. 1) recommended by Mašek[2] to prevent kinking of the tube. Recently Smolef's horizontal capillary[3] (Fig. 2) has been found very

suitable; the drop is regular, with no instability such as it has when it hangs from a vertical capillary. The curves are consequently very smooth and show no disturbances.

At first the only polarographic determinations possible were of substances that were themselves depolarisers, *i.e.*, that were reducible or oxidisable at the capillary electrode. Many organic compounds that are polarographically inactive may, however, be changed into depolarisers by one of the following methods, the first of which was introduced by Fieser *et al.*[4] for some ketosteroids (only ketones with conjugated double bonds are reducible). By applying Girard's T or D reagents the ketosteroids yield reducible hydrazones. Ketones are condensed—according to Zuman[5]—with secondary amines to ketoimines, which are reduced at positive potentials. Inactive aromatic compounds, such as benzene or phenobarbital, are nitrated; morphine—according to Baggesgaard-Rasmussen[6]—is converted by nitrous acid into a nitroso derivative. Other compounds are converted into reducible substances when treated with halogens, *e.g.*, methionine when treated with iodine. The growth factor leucovorin yields anodic and cathodic waves after oxidation by air in hydrochloric acid solution. For chlorates Koryta and Tengyl[7] found a suitable catalyst in an oxalic - sulphuric acid solution of titanous ions. These ions are oxidised by chlorate to the titanic state, but are instantly reduced electrolytically at the cathode. In turn they are oxidised again, and in this cyclic reduction produce a cathodic wave that gives a quantitative measure of the chlorate ions.

A number of methods have been found of producing the derivative curve, di/dE against E, which indicates the half-wave potentials better than the primitive current - voltage curve itself does. The best is that of Vogel and Říha,[8] who used a circuit in parallel with the electrolytic cell in which a condenser of about 3000 μF capacity and the galvanometer are placed.

The highest sensitivity is reached by the Barker[9] square-wave polarograph, in which the capacity current is entirely eliminated. This instrument records curves with a pen on paper and reveals traces of some cations in concentrations down to 10^{-8} M in the presence of a great excess of nobler constituents, *e.g.*, copper or tervalent iron.

The increasing demand for continuous and automatic control of solutions during industrial reactions directs attention to continuous-recording indicators. For these a most suitable reference electrode has been found in the dropping-mercury electrode, which in chloride solutions maintains the potential of the calomel electrode. It also keeps a constant potential in redox mixtures or strong oxidising agents such as ferric ions in dilute nitric acid; the reference electrode may be kept in a different solution from that being analysed and a slow flow has to be maintained. In this way the concentration of metallic ions or nitro bodies has been found from the recorded diffusion current at constant voltage. Such simple continuous recording is especially important when the concentration of carbon monoxide in the atmosphere has to be determined. The gas to be analysed for carbon monoxide is slowly passed over heated iodine pentoxide and the iodine liberated is absorbed in a dilute alkaline solution of bromate, which oxidises iodine to iodate.[10] A constant stream of a sodium sulphite solution removes the hypobromite and the atmospheric oxygen, and the final solution flowing around the dropping-mercury cathode and dropping-mercury anode produces the marked wave of iodate under the constant applied potential of 1·3 volts. Thus 0·01 to 0·0001 per cent. v/v of carbon monoxide can be recorded. For continuous recording solid electrodes are also suitable as reference electrodes, provided that under the passage of the current they do not yield solid products, *e.g.*, platinum electrodes surrounded by an acid solution of ferrous and ferric sulphates (0·1 N) or an alkaline solution of ferrocyanide and ferricyanide. By means of the latter the indicating dropping electrode records concentrations of cyanide ions up to 0·4 M.

For physiological researches into the amount of oxygen in living tissues, Šerák's vessel with the dropping electrode is suitable. As shown in Fig. 3, a piece of the tissue is placed on the cellophane, through which oxygen easily diffuses, and the loss of oxygen from the physiological solution is indicated by the current passing through the dropping-mercury cathode and silver-wire anode. Instead of the silver electrode another dropping-mercury electrode may be used. Before readings are taken the solution must be stirred.

Non-aqueous solutions are used whenever convenient. For example, for the determination of sulphur Harrison and Harvey[11] introduced ammonium acetate in glacial acetic acid. For the determination of sulphur dioxide in sulphuric acid 100 per cent. sulphuric acid may in practice be used. Non-aqueous solvents are applied mostly to dissolve organic

Fig. 3. Serák's vessel for measuring the uptake of oxygen

Fig. 4. Derivative oscillogram of penicillin G at the beginning of its decomposition in 0·01 *N* sulphuric acid

[To face page 190

substances. A suitable solvent for a number of hydrocarbons, including naphthalene, anthracene, styrene, stilbene and pyrene, is a mixture of 75 per cent. of dioxan with water or butanol. Another suitable mixture is light petroleum, benzene and methanol containing ammonium nitrate.

High polarographic accuracy (0·3 per cent.) is attained in polarometric titrations,[12] in which the limiting current of a depolariser serves as indicator. The construction of the titration graph is simple; two points well before the equivalence point and two after suffice for the drawing of two straight lines, the intersection of which gives the end-point. Sandberg[13] used sodium dipicrylaminate for the titration of potassium. A clever procedure was used by Langer[14] in titrations with fluoride ions. These ions bind the Th^{++++} or Al^{+++} ions until the nitrate wave, which appears only in the presence of those cations, disappears. Another ingenious method is the use of a "pilot ion" introduced by Ringbom.[15] When aluminium, calcium or magnesium are complexed by fluorides, the end-point is shown on the wave of a ferric salt, which is affected by the surplus of fluoride. Kolthoff[16] calls these titrations "amperometric," and uses his rotating platinum wire as the indicating electrode.

Since 1951, polarographic determinations have been coupled with chromatographic separations in quantitative analysis. Lewis and Griffiths[17] carried out their "paper-strip separations" and reached a high degree of accuracy in the analysis of ores. Later, Kemula[18] started his "chromatopolarography," for which he coupled the chromatographic column directly to the polarographic cell. These polarographic results may be photographically registered. In his separations he either elutes the components or uses the frontal method.

Finally a new trend in polarographic methods must be mentioned; this makes use of the cathode-ray oscillograph and may be called "oscillographic polarography." The cathode-ray oscillograph as developed some 20 years ago reached the sensitivity of a polarographic galvanometer. The charging current in oscillography is greater than the electrolytic current owing to the charging of each drop to the requisite potential being 300 times quicker. To avoid this inconvenience, cathode-ray polarographs are constructed to reproduce the polarographic current - voltage curve, but on one drop only. The procedure is first to polarise one drop of 7 or 8 seconds' duration at a steady voltage for 5 seconds, in which time the charging current becomes small and depolarisers of more positive potential are exhausted; then the voltage is raised through about 0·6 volt in 2 seconds and the peak due to the depolariser to be determined is shown by the luminous current - voltage curve. When the voltage is reversed, a more or less reversible anodic peak appears. High accuracy is claimed.

To avoid interference by the large charging currents in oscillography, the present author started with J. Forejt[19] in 1940 to study potential - time curves, V against t, with the dropping-mercury electrode. Alternating current from the 50-cycle mains at a constant amplitude controlled by a large resistance is so adjusted as to charge the dropping electrode in 0·01 second from zero to -2 volts and in the next 0·01 second back to zero, for which about 1 mA suffices. In this way the fluorescent screen shows curves that reveal depolarisers by time-lags or kinks in the potential - time curves and simultaneously denote their reversibility. Thus each depolariser is qualitatively characterised by two kinks, the cathodic and the anodic one, so that this sort of oscillography gives more information about quality than does polarography. One can increase the sensitivity somewhat by accelerating the time-base to 150,000 cycles per second, when the kinks give rise to luminous lines similar to those in an emission spectrum.

The sensitivity is, however, considerably increased by plotting the derivative curves dV/dt against t, or dV/dt against V. Mathematically it is clear that the short horizontal kink will appear on the derivative curve as an indentation. Physically the indentation is caused by the electrolytic current flowing in the opposite direction to the charging current. For this reason also the area of the indentation is a true measure of the quantity, as it gives the number of coulombs required for the electrolytic process.

For analytical measurements in oscillographic polarography with alternating current the "electronic polaroscope," which shows the curve of dV/dt against V, is most suitable. It might be applied in ore analysis, or in the determination of the purity of samples, especially of pharmaceutical products, such as vitamins, hormones and antibiotics, *e.g.*, penicillin G (Fig. 4).[20] Owing to the derivative adjustment, the oscillograms have a great resolving power, and can be used for distinguishing many organic isomers that would coincide polarographically. It also serves for detecting traces of noxious gases in factory atmospheres. For this purpose a simple tube with mercury at the bottom is used; this is filled with a suitable

electrolyte through which the air is sucked. Small amounts of carbon disulphide, hydrogen cyanide, hydrogen sulphide, sulphurous acid and even acetylene can be detected in this way.

To obtain stable figures, the trace must be suppressed for a constant time after the fall of each drop, and a screen with long afterglow must be used. The streaming-mercury electrode has been especially constructed to give stable oscillograms. In organic analysis the figures produced with the streaming electrode are simpler, as no side-reactions or consecutive reactions are possible.

In quantitative work empirical calibration curves have to be constructed by plotting the depth of the indentation against the concentration of the depolariser. This may be done conveniently with the luminous axis of Kalvoda,[21] a horizontal trace on the cathode-ray tube that can be moved vertically by adjusting a potentiometer.

A higher degree of accuracy can be attained with Kalvoda and Macku's[22] comparative titration procedure. In addition to the solution containing an unknown concentration of the component, a solution containing the same basic electrolyte is titrated until the oscillograms with the indentation overlap. Synchronised twin dropping-mercury electrodes are used and the two oscillograms are shown alternately on the screen every twenty-fifth of a second. In this way a 3 per cent. accuracy is possible. The sensitivity of the oscillographic method is often as high as that of polarography, revealing traces of depolarisers of the order of 10^{-5} moles per litre, especially when adsorptive substances are present *e.g.*, nitrobenzene or phenosafranine.

References

1. Heyrovský, J., and Shikata, M., *Rec. Trav. Chim. Pays-Bas*, 1925, **44**, 496.
2. Mašek, J., *Radiometer Polarographics*, 1952, **2**, 58.
3. Smoleř, I., *Coll. Czech. Chem. Comm.*, 1954, **19**, 238.
4. Wolfe, J. K., Hershberg, E. B., and Fieser, L. F., *J. Biol. Chem.*, 1940, **136**, 653.
5. Zuman, P., *Chem. Listy*, 1951, **45**, 65; 1952, **46**, 516, 521 and 599.
6. Baggesgaard-Rasmussen, H., Hahn, C., and Ilver, K., *Proc. Int. Congr. Pure Appl. Chem., London*, 1947, **11**, 125.
7. Koryta, J., and Tengyl, J., *Coll. Czech. Chem. Comm.*, 1954, **19**, 839.
8. Vogel, J., and Říha, J., *J. Chim. Phys.*, 1950, **47**, 5.
9. Barker, G. C., and Jenkins, I. L., *Analyst*, 1952, **77**, 685.
10. Novák, J. V. A., *Coll. Czech. Chem. Comm.*, 1955, **20**, 1076.
11. Harrison, S., and Harvey, D., *Analyst*, 1954, **79**, 640.
12. Heyrovský, J., *Bull. Soc. Chim. France*, 1927, **41**, 1224.
13. Sandberg, B., *Proc. 1st Int. Polarogr. Congr., Prague*, 1951, Part I, 227.
14. Langer, A., *Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 511.
15. Ringbom, A., and Wilkman, B., *Acta Chem. Scand.*, 1949, **3**, 22.
16. Kolthoff, I. M., *Trans. Electrochem. Soc.*, 1940, **78**, 191.
17. Lewis, J. A., and Griffiths, J. M., *Analyst*, 1951, **76**, 388.
18. Kemula, W., *Roczn. Chem.*, 1952, **26**, 281.
19. Heyrovský, J., and Forejt, J., *Z. phys. Chem.*, 1943, **193**, 77.
20. Heyrovský, J., *Coll. Czech. Chem. Comm.*, 1953, **18**, 739.
21. Kalvoda, R., *Ibid.*, 1955, **20**, 1503.
22. Kalvoda, R., and Macku, J., *Ibid.*, 1955, **20**, 255.

POLAROGRAPHIC INSTITUTE
CZECHOSLOVAK ACADEMY OF SCIENCES
PRAGUE

January 22nd, 1956

Analytical Applications of the Barker Square-wave Polarograph

Part II.* The Analysis of Copper-base Alloys and Steels

By D. J. FERRETT and G. W. C. MILNER

Further applications of the square-wave polarograph to the determination of alloying constituents in metallurgical products are described. This new technique is superior to conventional polarography for the analysis of copper-base alloys and steels. For example, the nickel and zinc contents of bronzes can be determined simultaneously in an ammonia - ammonium chloride base solution without any interference from copper. For the determination of lead, the copper ions must be complexed with cyanide and the lead peak can be recorded from an alkaline cyanide base solution. This procedure is useful for the determination of the lead content of samples of which only small quantities are available, *e.g.*, in the examination of copper alloys of historical importance.

Simple and direct procedures have been used for the determination of copper, tin and lead in steels by square-wave polarography. No chemical separations are necessary and the valency state of the iron is unimportant. The determination of chromium is possible after oxidation to the chromate state with ammonium persulphate. The chromate peak is recorded from an alkaline mannitol base solution.

The derivative nature of the square-wave polarograph[1] permits the determination of mixtures of elements that give mutually interfering steps in conventional polarography. Some advantages of this instrument in analysis have already been shown[2] and its application to the determination of alloying constituents in light alloys has been reported.[3] The major constituents of these alloys (aluminium or magnesium) are reduced at highly negative potentials at the dropping-mercury electrode and do not interfere with the determination of those alloying constituents that give steps at more positive potentials. With this type of alloy the square-wave polarograph can be used to determine directly minor constituents even when these are reduced at potentials more negative than those of other constituents present in much larger concentrations. For example, 0·01 per cent. of lead could readily be determined in aluminium alloys containing 4 per cent. of copper.

The application of the square-wave polarograph to the analysis of alloys with major constituents reducing near to zero applied voltage is a more difficult problem. Even so it has proved possible to use this technique for the determination of some of the constituents of steels and copper-base alloys. For most alloys direct methods have been developed, but in two instances it proved necessary to remove interference from the major alloying constituents by complex-ion formation.

THE ANALYSIS OF COPPER-BASE ALLOYS

Determination of zinc and nickel—

The determination of these elements by conventional polarography has been accomplished in a number of ways. Thus Hohn[4] and Milner[5] have determined nickel in alkaline cyanide solutions. Copper and zinc form such strong complexes under these conditions that they do not interfere with the nickel step. Zinc may be determined polarographically in an ammonia - ammonium chloride base solution only after the copper has been separated. Milner[6] applied a chemical method to do this, whereas Lingane[7] used controlled-potential electrolysis. In more recent work Pomeroy, White and Gwatkins[8] have shown that the zinc content of synthetic copper - zinc mixtures can be determined directly. Using the derivative circuit of Leveque and Roth, they obtained resolution of the zinc step for a zinc concentration range of 2 to 8 per cent.

* For particulars of Part I of this series, see reference list, p. 203.

Lingane's work has shown that the nickel and zinc steps are separated in ammonium hydroxide - ammonium chloride base solution. The separation is greatest (0·27 volt) in *M* ammonium hydroxide - 0·2 *M* ammonium chloride base solutions. As the sensitivity of the square-wave polarograph depends upon the degree of reversibility of the electrode reduction, we have studied base solutions with different ammonium hydroxide - ammonium chloride ratios to determine optimum conditions for the resolution and use of the nickel and zinc peaks. Results for the step heights in the base solutions studied are shown in Table I.

TABLE I

EFFECT OF THE BASE-ELECTROLYTE COMPOSITION ON THE HEIGHT OF THE NICKEL AND ZINC PEAKS

Base solution	*M* $NaClO_4$ 0·2*M* $HClO_4$	*M* NH_4OH 0·3*M* $(NH_4)_2CO_3$	*M* NH_4OH 6*M* NH_4Cl	*M* NH_4OH 3*M* NH_4Cl	*M* NH_4OH 0·2*M* NH_4Cl	*M* NH_4OH 0·1*M* NH_4Cl
Nickel peak height for 100 μg per ml	17	260	370	375	388	464
$E_{\frac{1}{2}}$, volts against the S.C.E.	−1·07	−1·07	−1·13	−1·12	−1·06	−1·05
Zinc peak height for 100 μg per ml	120	340	743	752	761	783
$E_{\frac{1}{2}}$, volts against the S.C.E.	−1·01	−1·25	−1·38	−1·36	−1·33	−1·30

In the sodium perchlorate - perchloric acid base solution the predominant ions present are $[Ni(6H_2O)]^{++}$ and $[Zn(4H_2O)]^{++}$. It can be seen that in ammoniacal solutions, in which the aquo ions are no longer the predominant species, the reversibilities of the two reductions are greatly increased, and the difference in behaviour between zinc and nickel becomes less pronounced. The reversibility of the electrode processes is greater in chloride than in carbonate solutions, and decreases in these former solutions with increasing chloride concentration. The differences here are not large, however, and as the nickel and zinc steps are most widely separated in 0·2 *M* ammonium chloride - *M* ammonium hydroxide solutions, this base solution was chosen for our investigation.

Copper gives two peaks with $E_{\frac{1}{2}}$ values of −0·25 and −0·51 volt against the S.C.E. from the ammonium hydroxide - ammonium chloride base electrolyte, these values being more positive than those for the nickel and zinc peaks. With alloys containing 0·1 per cent. of nickel, the copper concentration is a thousand times greater than that of nickel and the copper peak might mask the nickel peak. Experiments with solutions containing 5000 μg of copper per ml, however, showed that the recorder pen of the square-wave polarograph returned to the base line in the region of −0·7 volt against the saturated-calomel electrode (S.C.E.). It therefore proved perfectly feasible to determine nickel and zinc simultaneously in the presence of large amounts of copper. Fig. 1 shows a polarogram of a typical copper-base solution, the resolution of the zinc and nickel steps and the complete absence of any interference from the reduction of copper ions being indicated.

This technique appeared to be well suited to the direct determination of the nickel and zinc contents of bronzes. This type of alloy, however, contains several per cent. of tin, which is precipitated in a gelatinous form on the addition of the ammonium hydroxide - ammonium chloride base solution. Fortunately this element can be readily removed as its volatile bromide. The first step in the procedure for these alloys incorporates, therefore, an evaporation to dryness in the presence of bromine and hydrobromic acid. Further details follow.

Treatment of sample—Weigh 125 mg of sample, transfer it to a 125-ml conical beaker and add 10 ml of hydrobromic acid, sp.gr. 1·46. Warm to dissolve and then cool slightly when solution is complete. Add about 2 ml of analytical-reagent grade bromine and evaporate the solution to dryness, taking care to prevent loss by spattering in the later stages of the evaporation. Cool the beaker, and then add a further 5 ml of hydrobromic acid, repeating the evaporation to dryness. Cool the beaker and add 2 to 3 drops of concentrated nitric acid to the residue, followed immediately by about 5 ml of water. Boil the solution to remove bromine and evaporate to a low volume. Dilute this solution with about 5 ml of water and add ammonium hydroxide, sp.gr. 0·880, drop by drop until the solution is alkaline. Then dilute the solution to 100 ml with the *M* ammonium hydroxide - 0·2 *M* ammonium chloride base solution.

Transfer 5 ml of this sample solution to the polarograph cell, and add 0·1 ml of saturated sodium sulphite solution. Record a polarogram for the solution between —0·8 and —1·5 volts against the S.C.E. and compare the peak height with those obtained from standard solutions.

Preparation of calibration graphs—Weigh 110 mg of Specpure copper and dissolve it in 2 ml of diluted hydrochloric acid (1 + 1) and two drops of 100-volume hydrogen peroxide. Remove the excess of peroxide by boiling. Weight 62·5 mg of Specpure zinc and dissolve it in 2 ml of diluted hydrochloric acid (1 + 1) and dilute this zinc solution to 500 ml. Add 50 ml of the zinc solution to the copper solution and evaporate to a small volume. Add ammonium hydroxide, sp.gr. 0·880, until the solution is alkaline and dilute

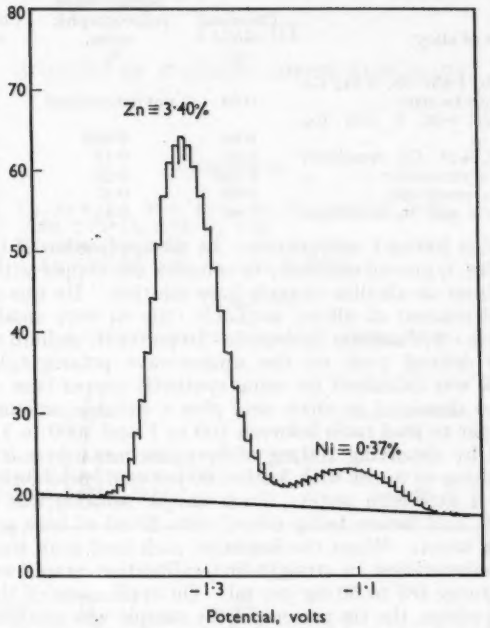

Fig. 1. Square-wave polarogram of bronze solution (100 mg per 100 ml), 1 *M* in ammonium hydroxide and 0·2 *M* in ammonium chloride

to 25 ml with *M* ammonium hydroxide - 0·2 *M* ammonium chloride solution. Transfer 5 ml of this standard solution to the polarograph cell, add 0·1 ml of sodium sulphite solution and record a polarograph as described above. Repeat the procedure, using 40, 30, 20, 10 and 0 ml of zinc solution, and plot the height of the zinc peak against the zinc concentration. The same technique was also used for the preparation of a calibration graph for nickel. Linear calibration graphs were obtained.

RESULTS—

Several typical copper-base alloys were analysed for nickel and zinc by the above procedure. A comparison of these results with the chemical figures is given in Table II.

The agreement shown here between the chemical values for these elements and those obtained with the square-wave polarograph indicates that this technique offers a very rapid and accurate method for the determination of nickel and zinc in bronzes, without pretreatment of the sample to remove copper as is normally necessary.

DETERMINATION OF LEAD—

The lead content of copper-base alloys cannot be determined directly by conventional polarography. Hence Lingane[7] removed copper from a solution of the alloy by controlled-potential electrolysis, the lead step then being recorded in a 4 *M* ammonium chloride - *M*

hydrochloric acid base solution. In another procedure, Milner[9] removed the polarographic interference of copper ions by adding potassium cyanide to the alloy solution to form non-reducible cuprocyanide ions. The lead step was then recorded from an alkaline cyanide base solution. Unfortunately, the copper and lead peaks from non-complexing base solutions are too close together to be satisfactorily resolved by the square-wave polarograph when the

TABLE II

DETERMINATION OF NICKEL AND ZINC CONTENTS OF COPPER-BASE ALLOYS

Composition of alloy, %	Nickel		Zinc	
	Chemical value, %	Square-wave polarographic value, %	Chemical value, %	Square-wave polarographic value, %
P, 0·25; Sn, 9·7; Pb, 1·83; Sb, 0·24; Cu, remainder (phosphor bronze)	0·04	not determined	1·86	1·91
Sn, 9·8; Pb, 0·41; Fe, 0·06; P, 0·05; Cu, remainder	0·09	0·093	2·53	2·54
Sn, 5·3; Sb, 0·2; Pb, 4·28; Cu, remainder	0·20	0·19	4·30	4·40
Sn, 10·4; Pb, 0·47; Cu, remainder	0·295	0·29	2·15	2·16
Sn, 5·8; Pb, 2·76; Cu, remainder	0·39	0·37	3·41	3·46
Pb, 2·47; Fe, 0·29; Cu and Sn, remainder	0·86	0·85	4·92	4·92

ratio of copper to lead is 100 to 1 and greater. In the application of the instrument to this determination, therefore, it proved necessary to complex the copper with cyanide ions and to record the lead peak from an alkaline cyanide base solution. By this means it was possible to determine the lead content of alloys, available only in very small amounts.

In a 0·6 *M* cyanide - 2 *M* sodium hydroxide - 10 per cent. sodium sulphite base solution lead produces a well defined peak on the square-wave polarograph. The quantitative behaviour of this peak was examined by using synthetic copper-base alloys, each prepared from 100 mg of copper dissolved in nitric acid plus a suitable amount of a standard lead solution to give a copper to lead ratio between 100 to 1 and 2000 to 1. The standard lead solution was prepared by dissolving 100 mg of Specpure metal in a few millilitres of nitric acid, and then evaporating to fumes with 5 ml of 60 per cent. perchloric acid. This solution was finally diluted to 1 litre with water. Each sample solution was evaporated to fumes with 5 ml of perchloric acid before being mixed with 25 ml of base solution and was then diluted to 100 ml with water. When the height of each lead peak was plotted against the corresponding lead concentration, a straight-line calibration graph was obtained for lead concentrations in the range 0·5 to 50 mg per ml. On application of this procedure directly to typical copper-base alloys, the tin present in the sample was precipitated as metastannic acid during the initial attack with nitric acid. The solution technique was therefore modified to include evaporation to dryness with brominated hydrochloric acid to remove tin from solution. Further details of the procedure follow.

Weigh out 100 mg of alloy and transfer it to a 125-ml conical beaker. Dissolve the sample in 10 ml of lead-free hydrochloric acid, sp.gr. 1·16. Then add 5 ml of hydrochloric acid saturated with bromine and evaporate to dryness. Repeat this treatment with a further 5-ml portion of brominated hydrochloric acid to remove the tin completely. Dissolve the residue in 5 ml of 60 per cent. perchloric acid and a few drops of nitric acid, sp.gr. 1·42, and then evaporate to fumes of perchloric acid. Add 25 ml of a 2·4 *M* potassium cyanide - 8 *M* sodium hydroxide - 40 per cent. w/v sodium sulphite solution and make the volume of the final solution up to 100 ml with water. Record the lead peak on a 5-ml portion of this solution. Then add a measured quantity of a suitable standard lead solution under the same base-solution conditions and record the lead peak. From the increase in the height of the lead peak, calculate the lead content of the alloy.

This procedure has been applied to the B.C.S. standard alloys, with the results reported in Table III.

In many types of lead-containing alloy the lead is known to be non-uniformly distributed throughout the material, and with such alloys it is necessary to take a large sample weight for analysis in order to obtain a representative figure for the lead content. In the work of proving the above procedure, therefore, it was considered desirable to take 100-mg quantities for analysis to offset any possibility of the lead being unevenly distributed. It

was realised, however, that because of the high sensitivity of the square-wave polarograph this analysis could have been accomplished on 5 to 10-mg samples. At the request of the Ashmolean Museum, Oxford, this technique was used for determining the lead content of bronze-age axeheads, samples from which were only available in small quantities. The procedure employed consisted in dissolving 10 mg of sample in 1 ml of concentrated hydrochloric acid and using 2-ml portions of brominated hydrochloric acid for removing the tin. After treatment with nitric acid, the solution was evaporated with 1 ml of perchloric acid to fumes. Then 2·5 ml of the base solution were added and the solution was finally diluted to 10 ml with water. The standard-addition technique was used as before. The results obtained for these samples are given in Table IV, and a typical polarogram is shown in Fig. 2.

TABLE III

ANALYSIS OF STANDARD COPPER-BASE ALLOYS

Nature of alloy	Composition, %	Lead	
		Chemical value, %	Square-wave polarographic value, %
Manganese brass B.C.S. sample No. 179	Cu, 58·8; Zn, 33·9; Mn, 1·03; Fe, 0·91; Al, 1·62; Sn, 1·75; Pb, 0·78; Ni, 1·01	0·78	0·81
Bronze "C" B.C.S. sample No. 207	Cu, 86·84; Sn, 9·80; Zn, 2·53; Ni, 0·09; Pb, 0·41; Fe, 0·06; Sb, 0·04; As, 0·05; P, 0·055	0·41	0·41

TABLE IV

ANALYSIS OF BRONZE-AGE AXEHEADS

Sample	Lead	
	Chemical value,[10] %	Square-wave polarographic value, %
1. Artefact from County Cavan, Ireland, 1927. 2848 ..	0·30	0·30
2. Artefact from Ciudad Real, Spain, 1927. 2004 ..	0·14	0·11
3. Artefact from Ireland, 1431. 2317	0·20	0·29

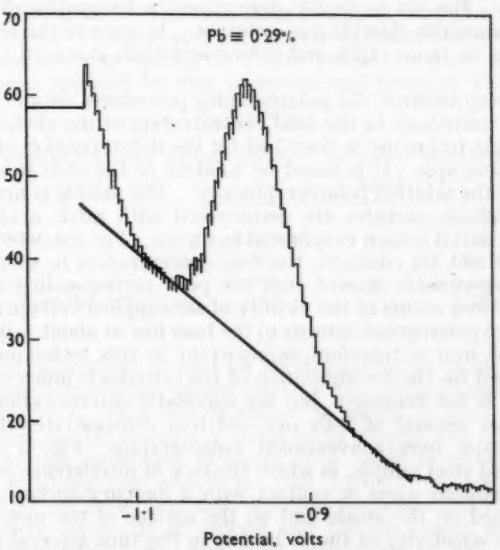

Fig. 2. Square-wave polarogram of a solution of Bronze-age artefact No. 3 (see Table IV) in 0·6 *M* potassium cyanide and 2 *M* sodium hydroxide

The chemical figures quoted in Table IV provide only a rough guide to the composition of the sample, as only very small sample weights were available for the standard chemical procedures. There is no evidence of segregation in these samples to explain the difference between the polarographic and chemical results. Four separate 10-mg portions of sample No. 3, for example, gave lead figures of 0·28, 0·31, 0·30 and 0·28 per cent., respectively.

THE ANALYSIS OF STEELS

Determination of copper, lead and tin

As the step due to the reduction of ferric iron starts from zero applied voltage, conventional polarography cannot be applied directly to the analysis of steel solutions if any oxidation has occurred. In some recommended procedures the iron interference is removed by chemical precipitation or by chemical reduction to the ferrous state. In other procedures the element to be determined is separated from other alloying constituents before being determined polarographically. Hence copper has been determined polarographically by Thanheiser and Maassen[11] and by Lingane and Kerlinger[12] after precipitation of iron (and chromium) with ammonia and with pyridine, respectively. In another method[13] copper and lead are determined simultaneously after most of the iron has been reduced to the ferrous state in a base solution containing hydrazine hydrochloride and sodium formate. In the solution of the sample decomposition of the carbides is carefully carried out with a saturated solution of potassium chlorate to prevent undue oxidation of iron.

Haim and Barnes[14] have determined lead in steels after dissolving the steel in 12 *M* hydrochloric acid in the absence of oxygen, this technique keeping the iron in the reduced state. This procedure is, however, unsuitable for the determination of copper, since this metal will not dissolve under the reducing conditions.

Allsopp and Damerell[15] have described a laborious procedure for the polarographic determination of tin in steels. The tin is precipitated (with copper and molybdenum) as the sulphide, which is separated and ignited to the oxide. The oxide residue is fused with potassium bisulphate, the melt is taken into solution with hydrochloric acid and this solution is then made alkaline with ammonium hydroxide. Small amounts of iron pass through to this stage and the ferric hydroxide precipitate acts as a carrier for the small tin precipitate. After solution of the hydroxide precipitate in acid, the iron is reduced to the ferrous state with hydroxylamine. The tin is finally determined polarographically from a *M* hydrochloric acid - 4 *M* ammonium chloride base solution. In spite of the above separations, this method is claimed to be more rapid and convenient than standard chemical methods for tin determination.

In addition to being indirect, the polarographic procedures require the addition of other reagents, which may contribute to the final concentration of the element being determined. In this section a simple technique is described for the determination of these elements with the square-wave polarograph. It is based on solution of the steel in acid, followed by the direct examination of the solution polarographically. The sample is first dissolved in hydrochloric acid, and insoluble carbides are decomposed with nitric acid. Perchloric acid is added to this solution and it is then evaporated to fumes. For the determination of a normal range of copper, lead and tin contents, the iron concentration in solution would be about 5000 mg per ml. Experiments showed that the peak corresponding to this concentration of fully oxidised ferric iron occurs in the vicinity of zero applied voltage and that the recorder pen of the square-wave polarograph returns to the base line at about −0·15 volt. The actual oxidation state of the iron is therefore unimportant in this technique and control of the amount of oxidant used for the decomposition of the carbides is unnecessary. The complete oxidation of the iron is not necessary, but the successful determination of lead and copper in the presence of this amount of fully oxidised iron demonstrates the superiority of the square-wave polarograph over conventional polarography. Fig. 3 shows a polarogram for a fully oxidised lead steel sample, in which the lack of interference from iron can be seen.

Ferric ions are reduced when in contact with a mercury surface, and in consequence a film is rapidly formed on the anode and on the surface of the mercury cup. This does not interfere with the sensitivity of the technique in the time-interval needed to record the polarograms. If the solution is allowed to stand in contact with mercury, however, the resistance of the film may be sufficient to prevent the efficient operation of the instrument. Details of the recommended procedures follow.

DETERMINATION OF COPPER—

Weigh 100 mg of steel and dissolve it in 10 ml of diluted hydrochloric acid (1 + 1) in a small beaker. When the dissolving action ceases, add 3 ml of nitric acid, sp.gr. 1·42, followed by 2 ml of 60 per cent. perchloric acid, and evaporate until fumes of perchloric acid appear. Cool the mixture slightly, wash down the sides of the beaker with a few millilitres of water and evaporate again until the perchloric acid fumes are seen. Cool, add 10 ml of hydrochloric acid, sp.gr. 1·16, and dilute to 100 ml with water.

Transfer 5 ml of sample solution to a polarograph cell, de-aerate with nitrogen and record the polarogram between −0·15 and −0·35 volt. Determine the copper concentration by the standard-addition technique, using a solution of copper in *M* hydrochloric acid. Results for the copper content of typical steels are given in Table V.

TABLE V

DETERMINATION OF COPPER IN DIFFERENT TYPES OF STEEL

Sample	Copper	
	Chemical value, %	Square-wave polarographic value, %
Carbon steel—		
0·03% carbon steel (A2) B.C.S. No. 150 ..	0·065	0·072
Low-alloy steels—		
B.C.S. No. 252	0·11	0·11
B.C.S. No. 255	0·24	0·23
B.C.S. No. 253	0·49	0·495
Alloy steels—		
B.C.S. No. 212	0·1–	0·11

DETERMINATION OF LEAD—

The amount of lead normally present in steels is very low, unless the lead has been deliberately added. The analysis of such steels is required, however, and the same procedure as that used for the copper determination suffices for the preparation of the solution. When 100-mg sample weights were taken for analysis, a significant variation in the results for lead on the same sample was observed. This behaviour is undoubtedly due to the heterogeneous nature of lead-bearing steels. This type of discrepancy disappeared, however, when 1-g samples were taken and the solution was diluted to 1 litre before the 5-ml aliquot was taken for analysis. Typical results for lead by this procedure are given in Table VI.

TABLE VI

DETERMINATION OF THE LEAD CONTENT OF LEAD-BEARING STEELS

Sample	Lead	
	Chemical value, %	Square-wave polarographic value, %
B.C.S. No. 212	0·28	0·285
Molybdenum steel (Mo, 0·275%)	0·15	0·16

DETERMINATION OF TIN—

If the square-wave polarograph is used for the determination of tin in steels, the normal technique[15] is considerably improved, as the need for the initial chemical separation of the tin is completely eliminated. This new procedure is similar to that described for copper and lead, and it permits the tin content of steels to be rapidly and accurately determined. In the development of this procedure, attempts were made to record the tin peak from solutions of the steel in 10 per cent. and 20 per cent. hydrochloric acid and in the 4 *M* ammonium chloride - *M* hydrochloric acid base solution recommended by Lingane.[16] Unfortunately, the peaks were somewhat ill defined and the tin results were inconsistent. In 5 *M* hydrochloric acid solutions, however, well defined peaks were obtained and these conditions were chosen for further investigation. Under these conditions satisfactory results were obtained for the tin content of a series of standard steels containing from 0·0005 to

0·26 per cent. of tin. The polarogram for B.C.S. steel No. 149 is shown in Fig. 4, the excellent separation of the copper and tin peaks being visible. In this example, it is interesting to note that the ratio of the copper and tin concentrations to that of the ferric iron is 1 to 5000 and 1 to 20,000, respectively. Further details of this determination follow.

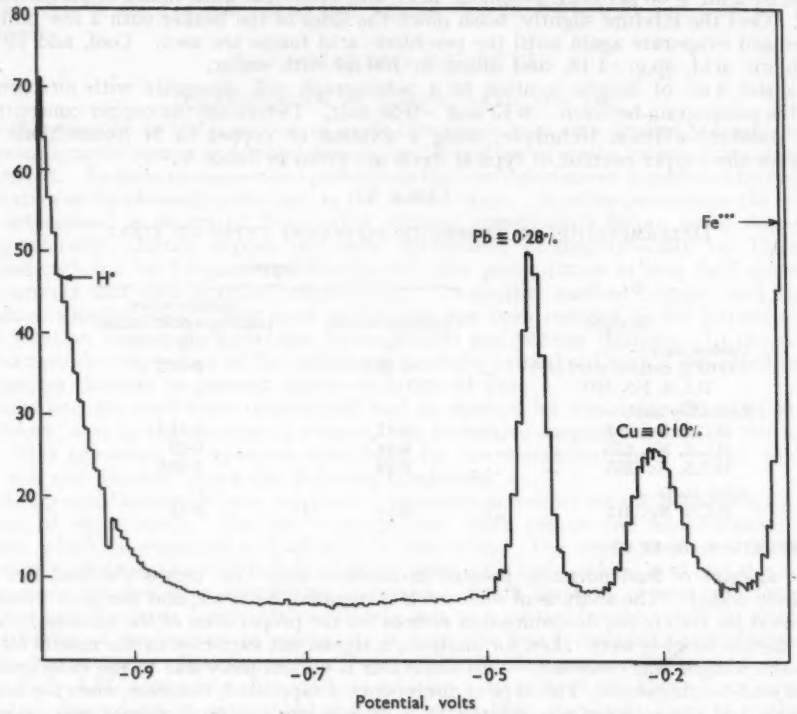

Fig. 3. Square-wave polarogram of a fully oxidised solution of B.C.S. lead steel (100 mg in 100 ml of *M* hydrochloric acid)

Take 100 mg of steel and dissolve it in 50 ml of hydrochloric acid, sp.gr. 1·16. Cool the solution and dilute it to 100 ml with water. Transfer 5 ml to the polarograph cell, de-aerate and record the tin step between —0·2 and —0·6 volt against the S.C.E. Determine the tin content by the standard-addition technique, using a tin solution in 5 *M* hydrochloric acid.

A linear relation for tin concentration against peak height was obtained. Typical results for the determination of tin in steels by this procedure are given in Table VII.

TABLE VII

POLAROGRAPHIC DETERMINATION OF THE TIN CONTENT OF STEELS

Sample	Tin	
	Chemical value, %	Square-wave polarographic value, %
B.C.S. No. 149	⩽ 0·002	0·00052
B.C.S. No. 218/1	0·040	0·037
B.C.S. No. 239/1	0·06–	0·052
S.G. 4676	0·066*	0·060
S.G. 4677	0·10*	0·10
S.G. 4678	0·17*	0·158
S.G. 4679	0·26*	0·28

* Chemical results by the Robertshaw - Bromfield procedure.[17]

These results show the ease and accuracy with which the tin content of steels can be determined with the square-wave polarograph. Unfortunately, any lead in the sample gives a peak coinciding with the tin peak. However, the lead content of steels is generally very small, with the exception of the lead-bearing type of steel. In the analysis of B.C.S. No. 149, this sample was examined for lead after the preliminary remova of tin as volatile stannic bromide. No peak was detected for this element.

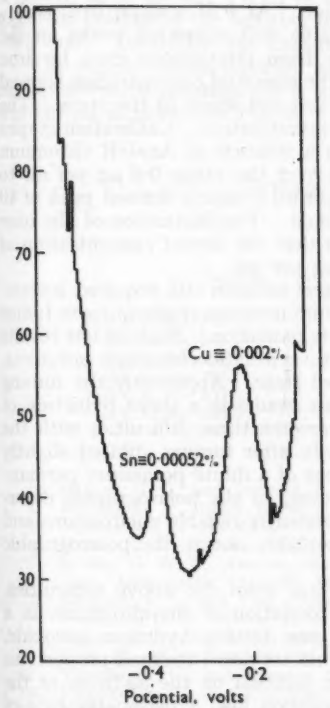

Fig. 4. Square-wave polarogram of a 1 mg per ml solution of B.C.S. steel No. 149 in 5 *M* hydrochloric acid

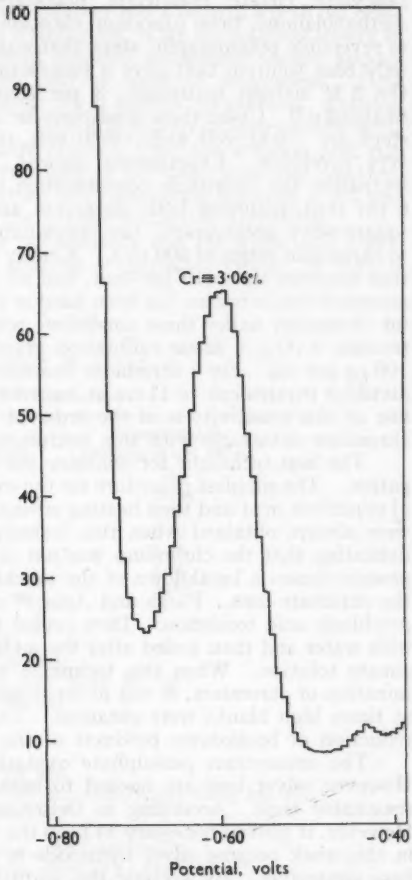

Fig. 5. Square-wave polarogram of a solution of B.C.S. steel No. 258 in 9 *M* sodium hydroxide containing 6 per cent. of mannitol, showing chromium peak

DETERMINATION OF CHROMIUM—

The most suitable step for the polarographic determination of chromium is that produced by the reduction of chromate ions from a strongly alkaline base solution. This step is reversible and its half-wave potential value is −0·85 volt against the S.C.E. in 0·1 to 1 *M* sodium hydroxide solutions. The chromate step has been used by von Stackelberg *et al.*[18] and by Thanheiser and Willems[19] in procedures for the determination of the chromium content of steels. The recommended methods, however, cannot be regarded as entirely satisfactory, because iron is precipitated by the base solution, and ferric hydroxide precipitates are noted for their ability to remove other elements from solution by adsorption. In later work Heyrovský[20] and co-workers have modified the technique to correct for the chromium

removed by the ferric hydroxide precipitate. The best approach to this problem, however, is to include a complexing agent in the base solution to prevent the precipitation of ferric hydroxide and to select solution conditions so that the polarographic reduction of the iron complex does not interfere with the chromate step. We have used this approach in applying the square-wave polarograph to the determination of chromium in steels.

A study of a sodium hydroxide base solution containing iron complexed with tartrate, salicylate, citrate, mandelate, malonate, glycollate, benzohydroxamate, triethanolamine, diethanolamine, tiron (disodium catechol-3:5-disulphonate) or sucrose showed the existence of reversible polarographic steps that unfortunately interfered with the chromate step. The only base solution that gave a satisfactory separation of the iron and chromium steps was the 3 *M* sodium hydroxide - 3 per cent. mannitol solution suggested by Reynolds and Shalgosky.[21] Under these conditions the half-wave potential values for the iron and chromium steps are −0·81 volt and −0·60 volt, respectively, against the S.C.E., but neither step is very reversible. Experiments showed, however, that the reversibility was improved by increasing the hydroxide concentration of the base solution. At 9 *M* sodium hydroxide - 6 per cent. mannitol both chromium and iron gave suitable well separated peaks on the square-wave polarograph, the chromium peak being free from interference even for iron to chromium ratios of 500 to 1. A study of the effects of the mannitol concentration showed that amounts up to 10 per cent. had no influence on the size and shape of the steps. The mannitol concentration has been fixed at 6 per cent. in this investigation. Calibration graphs for chromium under these conditions were prepared from a solution of AnalaR chromium trioxide (CrO_3), a linear calibration graph being obtained over the range 0·8 μg per ml to 100 μg per ml. For a chromium concentration of 0·2 μg per ml a clearly defined peak of 40 divisions (equivalent to 11 cm of recorder chart) was obtained. The fluctuation of the base line at this sensitivity is of the order of $\pm$ 5 divisions, so that the lowest concentration of chromium detectable with this instrument is about 0·03 μg per ml.

The best technique for oxidising the chromium in a steel solution still required investigation. The simplest procedure for the oxidation of chromium involves evaporating to fumes of perchloric acid and then heating strongly to give complete oxidation. Slightly low results were always obtained when this technique was applied to synthetic chromium solutions, indicating that the chromium was not in the fully oxidised state. Apparently the fuming process causes a breakdown of the perchloric acid, and this results in a slight reduction of the chromate ions. Parks and Agazzi[22] claim to have overcome these difficulties with the perchloric acid oxidation. They cooled the solution rapidly after fuming, diluted slightly with water and then boiled after the addition of a few drops of a dilute potassium permanganate solution. When this technique was applied, however, to the polarographic determination of chromium, it was never possible to obtain consistently reliable polarograms and at times high blanks were obtained. The blanks were problably due to the polarographic reduction of breakdown products of the perchloric acid.

The ammonium persulphate oxidation procedure is free from the above difficulties. However, silver ions are needed to ensure the complete oxidation of the chromium in a reasonable time. According to Oelschlägen,[23] the silver ions destroy hydrogen peroxide. However, it proved necessary to keep the amount of silver nitrate down to small proportions in this work because silver hydroxide is precipitated from solution on the addition of the base electrolyte. By keeping the acidity of the sample solution low, a reasonable rate of oxidation was obtained by using one drop only of a 0·15 per cent. solution of silver nitrate. The efficacy of the oxidation under these conditions when applied to steels was shown by the appearance of the red colour of oxidised manganese. The appearance of this colour indicates that the chromium has been completely oxidised. After the oxidation procedure, however, it proved necessary to destroy the remaining persulphate ions before recording the polarogram. It was observed that the decomposition of persulphate is much faster from strongly acid than from weakly acid solutions. After the oxidation of the chromium, the acidity of the sample solution was therefore increased and the solution was boiled to decompose the persulphate. The permanganate ions produced simultaneously with the chromate ions were reduced by the addition of one drop of concentrated hydrochloric acid to the warm sample solution. Then after this solution had been cooled, the base electrolyte was added and the polarogram recorded. Further details of the recommended procedure follows.

Weigh out 100 mg of sample and place it in a 150-ml conical beaker. Add 3 ml of 10 *M* sulphuric acid and 3 ml of water. Warm until the acid attack ceases, then add 2 or 3

drops of nitric acid, sp.gr. 1·42, and warm to decompose undissolved carbides. When solution is complete, evaporate to fumes of sulphuric acid, adding more sulphuric acid if chromic sulphate is precipitated. Cool and dilute to 10 ml with water.

Transfer 1 ml of sample solution to a 10-ml squat micro-beaker and add 10 *M* sodium hydroxide until a permanent precipitate just persists after vigorous stirring (usually 6 or 7 drops are required). Then add 0·1 ml of 10 *M* sulphuric acid and stir to redissolve the precipitate. Add 2 ml of 1·5 per cent. ammonium persulphate solution and 1 drop of a 0·15 per cent. silver nitrate solution. Then cover the beaker with a watch-glass, bring the solution slowly to the boil and simmer for 15 minutes (the red colour of oxidised manganese should appear after 5 to 10 minutes with steels containing 0·5 per cent. or more of manganese). Cool slightly, add 0·4 ml of 10 *M* sulphuric acid and simmer for a further 10 minutes, allowing the volume of the solution to decrease to about 2 ml. Then add 1 drop of hydrochloric acid, sp.gr. 1·16, and warm to discharge the permanganate colour. Cool the solution and run in rapidly with stirring 7·5 ml of a 12 *M* sodium hydroxide - 8 per cent. mannitol solution. Make the volume of the solution up to 10 ml and record the polarogram between −0·4 and −0·8 volt. Determine the chromium content by the standard-addition technique.

The typical results reported in Table VIII show the accuracy and the levels over which the square-wave polarograph is applicable in the analysis of low-alloy steels. A typical polarogram is shown in Fig. 5.

TABLE VIII

DETERMINATION OF CHROMIUM IN STEEL

Sample	Chromium	
	Chemical value, %	Square-wave polarographic value, %
B.C.S. No. 252	0·20	0·197
B.C.S. No. 254	0·53	0·50
B.C.S. No. 255	0·96	0·96
B.C.S. No. 256	2·34	2·36
B.C.S. No. 257	1·72	1·76
B.C.S. No. 258	3·07	3·06

REFERENCES

1. Barker, G. C., and Jenkins, I. L., *Analyst*, 1952, **77**, 685.
2. Ferrett, D. J., Milner, G. W. C., and Smales, A. A., *Ibid.*, 1954, **79**, 731.
3. Ferrett, D. J., and Milner, G. W. C., *Ibid.*, 1955, **80**, 132.
4. Hohn, H., *Z. Elektrochem.*, 1937, **43**, 127.
5. Milner, G. W. C., *Analyst*, 1945, **70**, 468.
6. ——, *Metallurgia*, 1947, **35**, 265; 307.
7. Lingane, J. J., *Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 429.
8. Pomeroy, P. R., White, R. A., and Gwatkins, G. H. R., *Metallurgia*, 1952, **46**, 157.
9. Milner, G. W. C., *Ibid.*, 1947, **36**, 287.
10. Case, H., *Man*, 1954, **54**, 18.
11. Thanheiser, G., and Maassen, G., *Arch. Eisenhüttenw.*, 1937, **10**, 441.
12. Lingane, J. J., and Kerlinger, H., *Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 77.
13. Nickelson, A. S., "Polarographic Estimation of Lead and Copper in Steel," *reported in* "Standard Methods of Analysis," United Steel Co. Ltd., Sheffield, 1951, p. 138.
14. Haim, G., and Barnes, W. C. E., *Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 867.
15. Allsopp, W. E., and Damerell, V. R., *Anal. Chem.*, 1949, **21**, 677.
16. Lingane, J. J., *J. Amer. Chem. Soc.*, 1945, **67**, 919.
17. Robertshaw, A., and Bromfield, G. C., *Analyst*, 1944, **69**, 340.
18. Stackelberg, M. v., Klinger, P., Koch, W., and Krath, E., *Forschungberichte Tech. Mitt Krupp, Essen*, 1939, **2**, 59.
19. Thanheiser, G., and Willems, J., *Mitt. K.-Wilh.-Inst. Eisenforsch*, 1939, **21**, 65.
20. Heyrovský, J., *Anal. Chim. Acta*, 1948, **2**, 533.
21. Reynolds, G. F., and Shalgosky, H. I., *Ibid.*, 1954, **10**, 273.
22. Parks, T. D., and Agazzi, E. J., *Anal. Chem.*, 1955, **27**, 416.
23. Oelschlägen, W., *Z. anal. Chem.*, 1955, **144**, 27.

NOTE—Reference 3 constitutes Part I of this series.

ANALYTICAL CHEMISTRY GROUP
ATOMIC ENERGY RESEARCH ESTABLISHMENT
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October 10th, 1955

Sucrose Loss from Ice-cream on Storage

By H. J. EVANS, W. KWANTES, D. C. JENKINS and J. I. PHILLIPS

(*Presented at the meeting of the Society in Cardiff on Saturday, December 10th*, 1955)

A probable cause of the diminution in content of sugar in certain samples of ice-cream during storage is attributable to the presence of the diplococcus *Leuconostoc mesenteroides*. This organism is well known for its ability to convert sucrose into dextran. The extent to which this change occurs will obviously depend upon many factors, the main ones being the time and temperature of storage and the number of organisms initially present.

Experiments show that a considerable change in the concentration of sucrose originally present in the ice-cream may occur during a few days' storage at 70° F, and even the temperature of a normal domestic refrigerator is not sufficiently low to prevent slow diminution of the sugar content.

When ice-cream was stored below 28° F, there was no evidence that changes in the concentration of sucrose occurred, but at this temperature only a very few trials were made. It is known that manufacturers of ice-cream store their products for many months in deep-freeze refrigerators without any apparent change in composition taking place.

It is recommended that samples of ice-cream intended for determination of their sucrose contents should preferably be analysed while they are still frozen solid and certainly should not have been kept more than a few hours at room temperature before the determination of sugar is begun.

Since the Food Standards (Ice-Cream) Order came into force in 1951, instances have been cited of changes occurring in the composition of samples of ice-cream during storage.[1] These changes affect primarily the sucrose content, which in some instances was found to diminish fairly quickly even when samples were stored in a refrigerator; in some samples stored at ordinary laboratory temperature the sucrose had almost disappeared. In view of the fact that the above-named and subsequent statutory orders require an ice-cream to contain at least 7·5 per cent. of sucrose, the need for investigating the phenomenon requires no emphasis. As a first step to this end, analyses were carried out on twelve samples before and after storage under various conditions.

Experimental

Each sample was divided into three parts, one part being examined immediately, a second portion after being kept for 3 days at 70° F and the third after being stored in a refrigerator at 36° F for 28 days. Conventional methods of determination were employed as under—

Fat—Röse - Gottlieb method (Draft British Standard).

Total solids—Drying to constant weight at 100° C.

Milk solids—Nitrogen determination. Only samples known to be free from gelatin were used.

Sucrose—Polarimetrically by Clerget inversion method, after clarification with zinc ferrocyanide. Only samples known to be free from starch were used.

Reducing sugar—The K value of the unstored series was determined on the solution as used for direct polarimetry, and any increase in the corresponding K value on the stored series was calculated to dextrose.

Results

The results obtained on the twelve samples are presented in Table I, II and III, corresponding to three different sources A, B and C.

Discussion of results

On considering the results presented in Table I, it will be seen that, although the content of fat had remained unchanged, the sucrose content of each sample had diminished. Of these

TABLE I

SAMPLES OF ICE-CREAM FROM SOURCE A

Method of storage	Fat, %	Sucrose, %	Increase in reducing sugar as dextrose, %	Sum of two previous columns	Milk solids, %	Total solids, %
Sample 1—						
Nil	11·33	13·10	—	13·10	10·24	36·70
3 days at 70° F	11·28	4·27	3·94	8·21	10·28	35·68
28 days at 36° F	11·35	1·59	4·87	6·46	10·05	35·93
Sample 2—						
Nil	5·63	15·11	—	15·11	8·24	30·64
3 days at 70° F	5·88	7·12	5·04	12·16	8·17	29·59
28 days at 36° F	5·63	12·01	0·60	12·61	8·13	29·86
Sample 3—						
Nil	10·21	12·78	—	12·78	10·87	32·98
3 days at 70° F	9·80	8·72	1·50	10·22	10·83	31·94
28 days at 36° F	9·54	7·43	0·41	7·84	10·54	32·06
Sample 4—						
Nil	5·22	13·34	—	13·34	12·78	33·88
3 days at 70° F	4·45	9·17	1·99	11·16	12·28	31·93
28 days at 36° F	5·42	12·40	Nil	12·40	12·29	32·52
Sample 5—						
Nil	11·72	13·36	—	13·36	10·23	37·37
3 days at 70° F	11·68	3·33	3·34	6·67	10·02	35·38
28 days at 36° F	10·41	0·94	2·23	3·17	10·19	36·24
Sample 6—						
Nil	11·46	14·25	—	14·25	12·27	38·68
3 days at 70° F	11·12	5·12	3·10	8·22	12·50	35·58
28 days at 36° F	11·31	5·46	2·23	7·69	12·34	37·40

TABLE II

SAMPLES OF ICE-CREAM FROM SOURCE B

Method of storage	Fat, %	Sucrose, %	Increase in reducing sugar as dextrose, %	Sum of two previous columns	Milk solids, %	Total solids, %
Sample 7—						
Nil	11·57	14·97	—	14·97	11·82	38·58
3 days at 70° F	11·62	8·98	2·25	11·23	11·76	38·68
28 days at 36° F	11·54	12·69	0·45	13·14	11·70	38·62
Sample 8—						
Nil	15·64	13·39	—	13·39	10·69	36·29
3 days at 70° F	12·84	8·75	1·20	9·95	10·69	33·88
28 days at 36° F	15·76	10·90	Nil	10·90	10·70	35·92
Sample 9—						
Nil	10·95	12·96	—	12·96	9·14	37·46
3 days at 70° F	10·56	6·71	2·44	9·15	9·33	37·00
28 days at 36° F	10·66	5·89	3·52	9·41	9·23	37·92

six samples, three showed greater loss of sucrose after 3 days at 70° F than after 28 days at 36° F, whereas in the other three samples from source A the reverse was true. Although the decrease in sucrose was accompanied by an increase in reducing sugar, the sum of the two did not equal the initial sucrose concentration. This apparent disappearance of sugar was most marked in sample No. 5 after storage for 28 days in a refrigerator, when the sucrose fell from 13·36 per cent. to 0·94 per cent. and the reducing sugar amounted to only 2·23 per cent. As neither the fat content nor the total solids had materially altered, it would appear that some of the sugar had been converted into some non-reducing non-volatile substance.

The same phenomenon of conversion of part of the sucrose with production of some reducing sugar and over-all loss of total sugar was manifested by storage of the three samples from source B (Table II). In this series No. 8, after storage for 3 days at 70° F, suffered

TABLE III

SAMPLES OF ICE-CREAM FROM SOURCE C

Method of storage	Fat, %	Sucrose, %	Increase in reducing sugar as dextrose, %	Sum of two previous columns	Milk solids, %	Total solids, %
Sample 10—						
Nil	5·59	10·66	—	10·66	7·55	26·10
3 days at 70° F	5·42	10·70	Nil	10·70	7·65	26·30
28 days at 36° F	5·23	9·98	Nil	9·98	7·60	26·75
Sample 11—						
Nil	9·28	13·27	—	13·27	12·29	35·53
3 days at 70° F	9·29	13·26	Nil	13·26	12·64	35·70
28 days at 36° F	9·20	13·02	Nil	13·02	12·45	36·31
Sample 12—						
Nil	10·19	10·70	—	10·70	14·29	35·28
3 days at 70° F	10·38	10·60	Nil	10·60	14·40	35·04
28 days at 36° F	10·26	10·71	Nil	10·71	14·13	35·79

a diminution in fat content very nearly equal to the loss in total solids. So far, this loss of fat has not been explained.

From Table III it will be seen that the samples of ice-cream from source C did not suffer any loss of sucrose on storage, neither was any reducing sugar produced. It was therefore evident that whatever was responsible for the changes in the sugar content of the samples from sources A and B was not present in the samples from source C.

It was noticed that the first nine samples had changed considerably after storage, the normal matt surface having become shiny, while the consistency became thick and gummy. In some samples the ice-cream could be pulled out into viscous threads, suggesting the formation of a long-chain polysaccharide. This, together with the fact that the total solids had remained constant, suggested that some of the sucrose had been converted into dextran.

A bacteriological investigation of the stored ice-cream with particular search for dextran-producing bacteria was therefore undertaken. This could only be carried out on the last six samples, as the earlier samples had been discarded by the time the enquiry was considered.

BACTERIOLOGICAL CONSIDERATION

Dextran-producing bacteria were first described by Pasteur in 1861. They are the cause of ropiness in wine and also caused the so called "frog spawn" in sugar factories during last century. The best known dextran-producing bacterium is *Leuconostoc mesenteroides*, which produces dextran from sucrose, but not from any other sugar. Its action on sucrose is complex and, in addition to dextran, fructose together with small quantities of mannitol and traces of acid are also produced.[2,3] The experiments described below showed that this organism could be isolated from a number of samples of ice-cream. Since *L. mesenteroides* survives pasteurisation,[4] it may well originate from any of the ingredients of the original mix or, alternatively, gain access by outside contamination.

BACTERIOLOGICAL ANALYSIS

The medium used for the isolation of *Leuconostoc* was a nutrient agar, containing 10 per cent. of sucrose, and having the following basic composition—

Lab Lemco (Oxoid)	1·0 per cent.
Peptone (Oxoid)	1·0 per cent.
Sodium chloride	0·5 per cent.
Powdered agar	1·5 per cent.

The ingredients were dissolved in tap water by heating in a steam steriliser, the mixture was filtered and the pH was adjusted to 7·6. The medium was then distributed into bottles, and sterilised by autoclaving at 10 lb pressure for 20 minutes. For use the agar base was melted in a steam steriliser, 10 per cent. of sucrose was added, the completed medium was heated in steam at 100° C for half-an-hour, and then approximately 15-ml portions were poured into Petri dishes and allowed to set. These plates were then inoculated each with a loopful of the ice-cream, and incubation was allowed to proceed at room temperature for

3 or 4 days. Colonies of *Leuconostoc* were identified by their large size and mucoid appearance. Pure cultures were obtained by taking single colony picks on to fresh plates of sucrose - agar medium.

Confirmation of the identity was made by the microscopic morphology of the bacteria, which were observed to be Gram-positive diplococci. All the isolated strains of *Leuconostoc* gave an acid reaction with glucose, maltose, lactose, sucrose, arabinose and xylose, and with mannitol there was no fermentation.

Dextran was isolated from all strains by growing them in 0·1 per cent. peptone water containing 10 per cent. of sucrose for 3 days at 20° C and then precipitating by adding a mixture consisting of 60 per cent. of ethanol and 40 per cent. of glacial acetic acid. After filtration and washing, a white gum was obtained, which, when hydrolysed with dilute hydrochloric acid, yielded glucose. The ability of the bacterium to produce dextran together with its ability to ferment pentoses constitutes supporting evidence in favour of the identification of the isolated organisms being *L. mesenteroides*. There was a difference in colonial morphology and also in the quantity of dextran produced by various strains of the organism.

L. mesenteroides was isolated in large numbers from samples No. 7, 8 and 9 after they had been stored, but none could be isolated from samples No. 10, 11 and 12. It was considered to be significant that those samples of ice-cream showing a reduction in sucrose content also contained large numbers of *L. mesenteroides*.

In a bacteriological examination of ten further samples of ice-cream, each taken from a different manufacturer, *L. mesenteroides* was found in five samples, which suggested that the occurrence of the organism is fairly widespread.

In order to determine whether *L. mesenteroides* would grow in ice-cream and cause loss in sucrose content, the following trial was conducted.

A single sample of ice-cream was equally divided and distributed into four 1-lb Kilner jars and all were sterilised by autoclaving. One of the sterilised bottles was inoculated with 1 drop of a 10 per cent. sucrose - peptone water culture, prepared overnight, of a strain of *L. mesenteroides* derived from ice-cream sample No. 7 and another bottle that had been sterilised was inoculated with one drop of a similar culture of a strain derived from ice-cream sample No. 8. These specimens, together with one of the remaining sterilised bottles containing ice-cream that had not been inoculated, were incubated at 70° F for 3 days. In the meantime the contents of the fourth sterilised Kilner jar and also the original ice-cream (not autoclaved) were submitted to chemical analysis. After 3 days, corresponding determinations of sugar and total solids were conducted on the incubated samples. The results are presented in Table IV and clearly demonstrate the effect of inoculation with *L. mesenteroides* and subsequent storage. They show a loss of sucrose, an increase in reducing sugar and no significant alteration in total solids, changes that are essentially similar to those presented in Tables I and II.

TABLE IV

EFFECT OF INOCULATION AND INCUBATION OF ICE-CREAM WITH *Leuconostoc mesenteroides*

Sample	Sucrose, %	Increase in reducing sugar as dextrose, %	Sum of previous two columns	Total solids, %
A	12·50	—	12·50	33·69
B	11·72	—	11·72	34·05
C	11·48	—	11·48	35·14
D	5·30	4·71	10·01	34·47
E	4·10	4·46	8·56	34·58

A, fresh ice-cream.
B, autoclaved ice-cream not inoculated, not incubated.
C, autoclaved ice-cream not inoculated, but incubated at 70° F for 3 days.
D, autoclaved ice-cream inoculated with *L. mesenteroides* strain 3 and incubated at 70° F for 3 days.
E, autoclaved ice-cream inoculated with *L. mesenteroides* strain 5 and incubated at 70° F for 3 days.

In a further series of trials the production of dextran by the action of *L. mesenteroides* on sucrose was demonstrated by inoculating 10 per cent. solutions of sucrose in 1 per cent. peptone water with two strains of the organism. These were analysed before and after incubation, and the results are shown in Table V. Similar changes to those occurring in

ice-cream took place, but, as the solutions were free from protein and fat, it was possible to precipitate and purify the dextran. From both, voluminous precipitates of a gummy nature were produced, which on being filtered off, washed, dried and re-precipitated yielded 5·36 per cent. and 3·10 per cent. of polysaccharide. In experiment C it will be noted that the amount of dextran obtained corresponded almost exactly to the loss of total sugar. Experiment D shows that no loss of sucrose or production of reducing sugar took place when the sucrose - peptone water medium was inoculated with an aerobic spore-bearing bacillus isolated from one of the ice-creams. It is of further interest to note that in experiment D no trace of a precipitate was discernible on adding the ethanol - acetic acid mixture after incubation, thus showing that no dextran was produced by this particular organism.

TABLE V

PRODUCTION OF DEXTRAN IN SUCROSE - PEPTONE WATER BY *Leuconostoc mesenteroides*

Sample	Sucrose, %	Reducing sugar as dextrose, %	Total sugar, %	Loss of total sugar, %	Dextran, %
A	9·33	Nil	9·33	—	Nil
B	1·99	4·20	6·19	3·14	5·36
C	2·61	3·60	6·21	3·12	3·10
D	9·33	Nil	9·33	Nil	Nil

A, sucrose - peptone water.
B, sucrose - peptone water inoculated with *L. mesenteroides* strain 3, kept at 70° F for 3 days.
C, sucrose - peptone water inoculated with *L. mesenteroides* strain 5, kept at 70° F for 3 days.
D, sucrose - peptone water inoculated with aerobic spore-bearing bacilli isolated from an ice-cream, kept at 70° F for 3 days.

The physical characteristics of the gums produced by the two strains of *L. mesenteroides* differed in that the dextran from strain 5 was flocculent and easily filtered off and washed, whereas that from strain 3 was a stringy precipitate that adhered, like a rubbery mass, to the side of the beaker. Such a precipitate would probably contain some impurities, and this may account for the fact that the dextran was recovered from the peptone water inoculated with strain 3 in slightly more than the theoretical maximum amount. When boiled with water, the two gums formed opalescent solutions that were strongly dextrorotatory; they did not reduce Fehling's solution, but, on being boiled under a reflux condenser with 10 per cent. hydrochloric acid for 1 hour, there was complete hydrolysis of the polysaccharides, to give a K value of 81·1 for hydrolysed "dextran 3" and 97·8 for hydrolysed "dextran 5." It seems reasonable to conclude that the conversion of sucrose into dextran by *L. mesenteroides* that occurs in sucrose solutions and artifically inoculated ice-cream can also take place in the commercial product.

REFERENCES

1. Wood, E. C., private communication.
2. Hucker, G. S., and Pederson, C. S., *N.Y. Agric. Exp. Sta. Bull.*, No. 167, 1931.
3. Giglio, D. M., and McCleskey, C. S., *J. Bact.*, **1953**, **65**, 75.
4. Liesenberg, C., and Zopf, W., *Zbl. Bakt.*, **1892**, **12**, 659.

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June 23rd, 1955

DISCUSSION

DR. J. H. BUSHILL said his interest in this subject was due to his being a member of a B.S.I. sub-committee dealing with the method of sampling ice-cream. The conclusions to be drawn from the paper had considerable bearing on the temperature at which samples of ice-cream should be stored while being transported to laboratories for analysis. That *L. mesenteroides* had been found in commercial samples of ice-cream was rather surprising; he would have thought that that organism, if present in the raw materials, would not survive pasteurisation. He mentioned experiments carried out on similar lines to those of the author's except that the storage temperatures were 12° F and 28° F. At the former temperature no change had been observed in the sugar contents over three months, and at 28° F only slight reduction was detected between the second and third months' storage. Strawberry ice-cream containing unpasteurised strawberry purée showed a slightly greater loss of sugar than did vanilla ice-cream under similar conditions.

DR. KWANTES replied that not only would *L. mesenteroides* survive pasteurisation but it would survive a temperature of 75° C for a quarter of an hour. This meant that once this bacterium had gained access to an ice-cream plant it was likely to remain there, because it was common practice to return ice-cream for re-processing; the plant was therefore being continuously re-infected. He added that if the ice-cream was aged for 2 to 3 days then *L. mesenteroides* might actually multiply in the ageing vats.

DR. BUSHILL then pointed out that the "ageing" of ice-cream mix was not really a factor in the multiplication of *L. mesenteroides*, as it was no longer the practice to age ice-cream for 2 to 3 days, but more usually for only a matter of hours.

DR. A. J. AMOS expressed an interest in the fact that the samples of ice-cream from source C behaved quite differently from those from sources A and B. Whereas in the last two decomposition of the sucrose into dextran and reducing sugars took place, no such deterioration occured in the first. He asked whether the authors had any information as to whether the difference was due to samples from source C having a much lower initial contamination with *L. mesenteroides*, to the C samples having been subjected to a processing that killed many of the micro-organisms, or to samples A and B having been subjected to conditions, such as protracted ageing, that led to a significant increase in the initial microbial population.

DR. KWANTES replied that the samples from source C came from producers who sold small quantities of ice-cream, whereas A and B were larger concerns. The producers grouped as source C would probably make separate batches of ice-cream and were unlikely to return any of their product for re-processing. The small producer would therefore not continuously re-infect his plant.

MR. GEORGE TAYLOR said that Dr. Kwantes's cultivations were mostly at 70° F, but he had found loss of sucrose at lower temperatures. He asked what proportional loss of sucrose would Dr. Kwantes expect in an ice-cream kept in an ordinary domestic refrigerator for 72 hours—say from Friday to Monday. He also asked what the sensitivity of *L. mesenteroides* was to inhibition by other micro-organisms.

DR. KWANTES replied that the experiments showed that a loss of sucrose did occur in samples kept in an ordinary domestic refrigerator and added that the extent would probably depend on the numbers of *L. mesenteroides* present. Some of his experiments had shown that *L. mesenteroides* would grow on sucrose - agar plates in a normal domestic refrigerator. He found that a growth equivalent to three days at 70° F took about three weeks in the refrigerator. *L. mesenteroides* was often found in ice-cream in the presence of other organisms and would therefore have to compete with them; the extent of any sucrose loss would no doubt be greatest if *L. mesenteroides* was present in pure culture.

MR. HYDE agreed with Dr. Bushill that an ageing time of 2 to 3 days was no longer general practice. He had carried out some experiments and had found a reduction of sucrose and other sugars in ice-cream samples stored for 1 to 2 days. He had measured a reduction in sucrose content from 14·8 to 14·4 per cent. and in total reducing sugar (as lactose) from 5·4 to 5·2 per cent. on overnight storage at room temperature, and with a fruit ice-cream a reduction in sucrose content from 12·2 to 10 per cent. and in total reducing sugar from 8·8 to 8 per cent. on storage for 48 hours. He had on one occasion found a loss of sucrose, but bacteriological analysis had given a zero plate count; he wondered if this could be explained.

DR. KWANTES said that as *L. mesenteroides* would not grow on nutrient agar, a zero plate count on nutrient agar did not necessarily mean that *L. mesenteroides* was not present in the ice-cream. A sucrose - agar medium would show whether they were present or not.

MR. D. D. MOIR said he thought there must be a critical temperature for the growth of *L. mesenteroides* in ice-cream. In his experience, if samples were delivered to the laboratory within two or three hours of sampling, when they were still cold, and then placed immediately in a refrigerator kept at 33° F, no loss of sucrose occurred for one, two or even three weeks after receipt. The temperature in a domestic refrigerator could rise to 40° F or more, when the loss of sucrose might become significant.

DR. KWANTES said that there was no doubt a critical temperature above which ice-cream might suffer loss of sucrose. Further experiments would be required to determine what this temperature was.

MR. O. NEAVE suggested that the freezing unit of an ordinary domestic refrigerator should be quite suitable for storing samples of ice-cream for analysis.

MR. TAYLOR asked what the loss of sucrose was over a period of, say, 6 hours for samples stored with solid carbon dioxide in a wooden insulated box.

DR. KWANTES replied that he had had no experience of transporting ice-cream samples with solid carbon dioxide, but that virus specimens were transported with "dry ice" and remained frozen solid for up to 24 hours in the post.

MR. M. C. FINNIEAR said that the authors had stated that they had used zinc ferrocyanide to clear the ice-creams for polarimetry. He asked why some ice-creams were difficult to clear by any of the recognised clearing agents.

MR. EVANS replied that they had found ice-creams that had become dextranised (or gummy) difficult to clear. In some instances optical reading was impossible and sucrose had been determined by using Fehling's solution (Lane and Eynon's method).

DR. T. G. MORRIS asked what was the source of *L. mesenteroides*.

DR. KWANTES replied that Bergey's "Manual of Determinative Bacteriology" stated that the natural sources were milk and plant juices.

DR. BUSHILL then said that as milk was used in the preparation of ice-cream this might be the source of the *L. mesenteroides* for the product.

He went on to remind the meeting that should the temperature of ice-cream rise above 28° F, it must not be sold or re-frozen without re-pasteurising, and, in view of that, he asked whether ice-cream whose temperature had exceeded 28° F could still be termed "ice-cream." He suggested that the answer to that question might influence the decision as to the temperature below which samples should be maintained before analysis.

MR. EVANS replied that if the sample was in a liquid state when received for analysis, he would still consider it as a sample of ice-cream (under the Food and Drugs Act).

MR. R. G. MINOR asked whether there were any chemical analytical methods available for the recognition and determination of dextran produced in ice-creams of unknown composition, with a view to ascertaining the approximate original composition.

MR. EVANS replied that, although they had not yet been able to determine the amount of dextran in an altered ice-cream, a close approximation to the original composition could be obtained from the difference between the sum of the fat and milk solids other than fat, and the total solids as actually determined. Starch, if present, had to be allowed for.

Inter-laboratory Trials on the Determination of Quartz in Dusts of Respirable Size

Report from Panel 2 of the Dust Sub-Committee of the Medical Research Council's Industrial Pulmonary Diseases Committee

BY G. NAGELSCHMIDT (*Secretary of Dust Sub-Committee*)

In order to test the reliability of methods for the quantitative determination of quartz in mixtures with silicates, a series of inter-laboratory trials was made, under the auspices of the Medical Research Council's Sub-Committee on Dust. Several chemical methods, X-ray diffraction and differential thermal analysis were used, and from twelve laboratories nineteen sets of results on seven samples were obtained. A rational analysis variant, described in an Appendix, showed the best reproducibility amongst the chemical methods. After allowance had been made for variability of standards used in different laboratories, X-ray analysis gave nearly as good results as the best chemical method, but differential thermal analysis showed a wider scatter of results. Even when a standard quartz sample was first analysed by different laboratories and allowance for variation of results was made, the results by the best chemical and X-ray methods varied by plus or minus seven units of quartz percentage, *i.e.*, 16 ± 7 per cent. or 70 ± 7 per cent. Individual laboratories produced better results by various methods, including differential thermal analysis.

DEFINITION—Quartz is a mineral characterised by its chemical composition (100 per cent. SiO_2) and by its crystal structure, which leads to certain physical properties; of these the X-ray pattern, the refractive indices for light and the reversible thermal inversion from the α to the β form have been used for analytical purposes. Quartz and "free silica" are often used as equivalent terms, but because the latter is ambiguous, the term "quartz" is used in this paper.

THE accurate determination of quartz in the presence of silicate and other minerals is a problem of great importance in silicosis research and control. There is a considerable number of chemical methods to be found in the literature, and quantitative physical methods such as X-ray diffraction and differential thermal analysis have been described. A recently developed optical method "dispersion staining" is a further possibility (Crossmon[1] and Thaer[2]).

In order to assess the accuracy and reproducibility of the different techniques, an inter-laboratory trial was arranged under the auspices of the Sub-Committee on Dust of the Medical Research Council's Industrial Pulmonary Diseases Committee. Seven samples of mixed dusts of respirable size, with a quartz content ranging from 100 to 0 per cent., were analysed by different methods in seven laboratories in this country and five laboratories abroad (France, Holland, Italy, South Africa and U.S.A.). The dusts had been chosen to represent the mineral matter found in coal-mine dusts and lung residues of coal miners, but similar materials occur in pottery, slate and other dusts.

Experimental

Description of the samples and of their preparation—

The participating laboratories are represented by code letters A to M. All samples were prepared at, and distributed by, laboratory A. Three pure minerals were chosen, namely quartz, kaolinite and muscovite, and suitable-size fractions were prepared by sedimentation methods. Four mixtures were made from the sized minerals. Code numbers of all samples and composition of the mixtures are given in Table I.

Table I

Quartz analysis samples

Minerals below 5μ Stokes's diameter

Sample or mixture	Composition of samples: Quartz X1072, %	Kaolinite X1876, %	Muscovite X2431, %
X1072	100	0	0
X1876	0	100	0
X2431	0	0	100
X2478	0	28·5	71·5
X2479	14	24·6	61·4
X2480	48·4	14·8	36·8
X2043	66·7	33·3	0

Quartz, X1072—This was prepared from a commercial sample of fine pure Belgian glass sand, which was found on chemical analysis to contain 99·7 per cent. of SiO_2. A fraction below 5μ equivalent diameter was prepared by repeated sedimentation in distilled water, followed by removal of water by centrifuging.

The size distribution of sample X1072 was determined by the gravimetric sedimentation method (pipette analysis) and by microscope counts at laboratory A. The results are given in Table II, in which the mass distribution calculated from the microscope counts is given. It will be seen that Stokes's diameters correspond fairly well to 1·5 times larger projected diameters. Chemical analyses of X1072 made by several laboratories are given in Table III.

Table II

Size distribution of X1072 (results from laboratory A)

Pipette analysis: Size, μ	Pipette analysis: Mass below size, %	Microscope count converted to mass distribution: Size, μ	Microscope count converted to mass distribution: Mass below size, %
1	27·5	1·5	23
2	53·0	3	56
3	78·0	5	86
5	98·5	7	99
7·5	100·0		
10	100·0		

Kaolinite, X1876—A commercial sample of china clay from Cornwall was chosen. The approximate pipette-analysis specification for this material is 50 per cent. of the mass below 1μ and 80 per cent. below 2μ Stokes's diameter. This type of clay may contain 5 to 10 per

TABLE III

CHEMICAL ANALYSIS OF X1072

	Laboratory A	Laboratory B	Laboratory C	Laboratory D	Laboratory E
SiO_2 (by hydrofluoric acid method), %	98·5	98·3	98·6	98·5	99·3
Ignition loss, %	0·5				
Residue, %	1·1				
Total, %	100·1				
SiO_2 (gravimetric method), % ..		101·2			

cent. of mica, but usually does not contain more than 0·2 per cent. of quartz. Pipette analysis of X1876 at laboratory A gave—

Size, μ	0·5	1	2	3	5
Mass below size, % ..	16	53	81	85	91

A partial chemical analysis by laboratory D gave: $SiO_2 + H_2O$, 60·3 per cent.; $Al_2O_3 + K_2O$, 39·7 per cent. This is close to the theoretical composition of kaolinite, *i.e.*, SiO_2, 45·5 per cent.; Al_2O_3, 39·6 per cent.; H_2O, 13·9 per cent.

Muscovite, X2431—A commercial sample of ground muscovite from India was used as starting material. A fraction below 4μ equivalent diameter was prepared by sedimentation in water, followed by centrifuging.

Size distribution of X2431, determined by pipette analysis at laboratory A, gave—

Size, μ	0·5	1	2	3	5
Mass below size, % ..	5	12	44	70	87

X2043, a mixture of 66·7 *per cent. of quartz and* 33·3 *per cent. of kaolinite*—A 100-g portion of X1072 and a 50-g portion of X1876 were weighed into a wide-mouth 2-litre glass bottle. Then 800 ml of benzene were added and the bottle was shaken for 3 hours. The contents of the bottle were poured into a porcelain dish, and benzene was used to wash the bottle. Most of the benzene was poured off and the rest was removed by evaporating at 60° C. The dry material was broken up, well mixed on glazed brown paper and placed in a stock bottle, from which small bottles for distribution were filled.

X2478, a mixture of 71 *per cent. of muscovite and* 29 *per cent. of kaolinite*—A 100-g portion of X2431 was mixed with a 40-g portion of X1876. The mixing technique was the same as that used for X2043.

X2479, a ternary mixture containing 14 *per cent. of quartz*—An 86-g portion of the mixture X2478 was mixed with a 14-g portion of X1072. The mixing technique was the same as described above.

X2480, a ternary mixture containing 48·4 *per cent. of quartz*—A 30-g portion of mixture X2479 was mixed with a 20-g portion of X1072. The mixing technique was the same as used previously.

RESULTS OF TRIALS

CHEMICAL DETERMINATION OF QUARTZ—

The literature on chemical determinations of quartz has recently been reviewed by Durkan[3] and Talvitie,[4] who each introduced new variants. The first analyses on X1072 quartz were carried out in four laboratories. Macro and semi-micro Shaw methods (H_2SO_4), three modifications of the Trostel - Wynne[5] method ($NaHSO_4$) and two modifications of Durkan's method (H_3PO_4) were used. These differ mainly in the choice of the attacking reagent, given in brackets above, and in details of alkali treatment for removing amorphous silica.

The results showed considerable discrepancies between different methods and, for one and the same method, between different laboratories. The most promising appeared to be a semi-micro variant (Shaw - Skinner, see Appendix, p. 217) of the "rational analysis" described by Shaw[6] and the single-acid Durkan[3] techniques. Later trials with further samples led to the elimination of the Durkan and the retention of the Shaw - Skinner method. As this method is empirical, working conditions must be fixed in considerable detail; a full description, as agreed by the participating laboratories, is given in the Appendix.

A number of other laboratories became interested and took part in this work. Laboratories G, H and J used their own modifications of the Talvitie or Durkan methods.

The final values by the Shaw - Skinner method obtained for all samples are shown in Table IV. The results for X1072 vary between 86 and 92 per cent., with a mean value of 90 per cent. Results of five laboratories by three different phosphoric acid methods are given in Table V. Three of these show good agreement at low values, the remaining two at higher values.

TABLE IV

CHEMICAL DETERMINATION OF QUARTZ BY SHAW - SKINNER METHOD

	Quartz determined by				
	Laboratory D, %	Laboratory E, %	Laboratory F, %	Laboratory A, %	Mean, %
X1072 Quartz	92	88	92	86	89·5
X1876 Kaolin	2	8	3	4	4·2
X2431 Mica	2	4	0	14	5·0
X2478 (no X1072) ..	9	1	0	4	3·5
X2479 (14% of X1072)	14	15	10	15	13·5
X2480 (48% of X1072)	44	43	44	46	44·2
X2043 (67% of X1072)	58	60	61	62	60·2

TABLE V

CHEMICAL DETERMINATION OF QUARTZ BY PHOSPHORIC ACID METHODS

	Quartz determined by					
	Laboratory H, %	Laboratory A, %	Laboratory J, %	Laboratory D, %	Laboratory G, %	Mean, %
X1072 Quartz	78	78	91	78	93	83·6
X1876 Kaolin	5	5	—	1	5	4
X2431 Mica	—	7	1	5	1	3·5
X2478 (no X1072) ..	1	5	1	7	1	3
X2479 (14% of X1072)	14	8	15	13	14	12·8
X2480 (48% of X1072)	34	24	46	40	44	37·6
X2043 (67% of X1072)	65	63	61	56	62	61·4

X-RAY DIFFRACTION ANALYSES—

X-ray diffraction analysis, especially by Geiger - Müller diffractometer techniques, is increasingly used for quartz determinations. Again, a number of laboratories not represented in the original Medical Research Council Group participated in the work at a later stage, and full results from six laboratories are assembled in Table VI. All laboratories, except A,

TABLE VI

DETERMINATION OF QUARTZ BY X-RAY DIFFRACTION ANALYSIS

	Quartz determined by							
	Laboratory A, %	Laboratory K, %	Laboratory M, %	Laboratory F, %	Laboratory L, %	Laboratory G, %	Laboratory J, %	Mean, %
X1072 Quartz	80	91	73	86	87	75	98	84
X1876 Kaolin	< 0·5	—	—	1·5	—	—	0	1
X2431 Mica	0	0	—	—	0	0	—	0
X2478 (no X1072) ..	0·5	0	0	—	0	0	—	0
X2479 (14% of X1072)	10	15	10	11	13	16	—	12
X2480 (48% of X1072)	37	44	37	40	36	40	—	39
X2043 (67% of X1072)	50	64	51	55	61	48	64	56

used calcium fluoride as internal standard; K, F and J photographically and M, L and G with Geiger - Müller diffractometers. Laboratory A used an external-standard technique on a specially built Geiger - Müller equipment. Laboratory J used calcium fluoride, but reported results only in the absence of mica.

The results show a considerable spread and they have therefore been recalculated in Table VIa for the three mixtures containing quartz on the basis of assigning a value of 100 per

cent. to the sample X1072. It will be seen that the range of results has been considerably reduced by this procedure. All X-ray methods are calibrated on some standard, which is taken as 100 per cent. quartz. As the values for X1072 range from 73 to 98 per cent., it follows that the standards used in different laboratories did vary.

TABLE VIa

DETERMINATION OF QUARTZ BY X-RAY DIFFRACTION ANALYSIS

(CORRECTED FOR X1072 = 100 PER CENT.)

	Quartz determined by							
	Laboratory A, %	Laboratory K, %	Laboratory M, %	Laboratory F, %	Laboratory L, %	Laboratory G, %	Laboratory J, %	Mean, %
X2479 (14% of X1072)	13	16	14	13	15	21	—	15
X2480 (48% of X1072)	46	48	50	45	41	53	—	47
X2043 (67% of X1072)	62	70	69	63	70	64	65	66

DIFFERENTIAL THERMAL ANALYSIS—

Three laboratories only used differential thermal anslysis. In laboratory E peak heights and peak areas were measured as against peak areas only by laboratories H and C. Table VII shows the results as reported and also the results corrected for X1072 having a value of 100 per cent.

TABLE VII

DIFFERENTIAL THERMAL ANALYSIS FOR QUARTZ

	Quartz determined by			
	Laboratory E			
	by peak-height measurement, %	by peak-area measurement, %	Laboratory H, %	Laboratory C, %
X1072 Quartz	60 (100)	79 (100)	77 (100)	71 (100)
X1876 Kaolin	—	—	< 0·5	2·8
X2431 Mica	—	—	1	—
X2478 (no X1072) ..	—	—	0·7	—
X2479 (14% of X1072)	5 (8·5)	9 (11)	9·5 (12·5)	16·2 (22·6)
X2480 (48% of X1072)	24 (40)	31 (39)	33·5 (44)	49·5 (70)
X2043 (67% of X1072)	31 (52)	40 (50)	50 (63)	66 (93·3)

It will be seen that peak-area measurements give higher results than peak-height measurements. The corrected results of laboratory H and the uncorrected results of laboratory C correspond closely to the amounts of sample X1072 given.

DISCUSSION

CHOICE OF SAMPLES—

As the trials were concerned with estimation of silicosis hazard, the size range of interest was roughly below 5μ. By far the largest numbers of men affected by lung disease caused by dust in this country are found in the coal-mining industry. Coal-measure rocks are composed mainly of mica, quartz and kaolin, with variable minor amounts of other minerals. Slate and pottery dusts also consist largely of the same constituents. These considerations controlled the type and size grading of the samples used for the analytical trials. In what follows a few general comments will be made on the different methods used, before the results obtained are discussed.

COMMENTS ON METHODS—

Chemical method—A determination of total silica in a dust sample can be carried out with great accuracy by any competent analyst. In contrast to this, a chemical determination of quartz is a much more uncertain task because it is not based on simple chemical laws. What is in fact done in the Shaw - Skinner method, described in the Appendix (p. 217), is to attempt to dissolve the silicates in hot concentrated sulphuric acid without attacking the quartz. As many silicates under these conditions release their silica in an amorphous

form insoluble in the hot acid or in the washings, this amorphous silica has to be kept in solution by treating the decomposed sample with dilute alkali.

Quartz itself is to a slight extent attacked by the hot acid and to a greater extent by the dilute-alkali treatment. How much it is attacked will depend on the amount of quartz in the sample and especially on its size. As the quartz gets smaller, its specific surface increases and hence its attackability increases. This is illustrated by the results shown in Table VIII for carefully sized quartz fractions, which were subjected in duplicate to the method described in the Appendix. Thus the attackability leads to an error that will depend on amount and size of the quartz. But this is not all. In the Shaw - Skinner method the residue from the combined acid and alkali attack is ignited and weighed (first residue). It is composed of quartz and perhaps some other undecomposed material. It is next treated with hydrofluoric acid, when a second, usually smaller, residue may be obtained. This represents the non-siliceous portion of previously undecomposed material. It has been found empirically that if twice the weight of the second residue is subtracted from the first residue, the balance represents the quartz in the sample. This holds for mine dusts, because the mica and kaolin in the dusts contain roughly 50 per cent. of SiO_2 after calcination. If abrasives were tested and the dusts consisted of quartz and corundum, the Shaw - Skinner method would turn out absurd results, because all the corundum would be found in the second residue and the same correction would give large negative results for the quartz content.

TABLE VIII

FREE SILICA CONTENT OF SAMPLES OF GRADED QUARTZ DETERMINED BY THE SHAW - SKINNER METHOD

(RESULTS BY LABORATORY D)

Size, μ	Free silica, %	
< 2	87·3	86·0
2 to 4	88·7	88·6
4 to 8	94·4	94·7
10 to 20	98·0	97·6
20 to 40	95·9	95·9

Thus it must be recognised that the Shaw - Skinner method is empirical in the sense that it is sensitive to details of operation, that increasingly low results will be found as the grain size of the quartz decreases and that it should not be used uncritically on any material to be analysed.

X-ray diffraction—In theory, X-ray diffraction technique is very suitable for quartz determinations. Quartz has a distinct diffraction pattern with many well spaced lines of differing intensity, and for this reason quartz is often used to calibrate diffraction cameras. In quantitative analysis of mixtures it is customary to use an internal-standard technique to correct for absorption and other effects. The development of Geiger - Müller counter diffractometers has greatly facilitated and speeded up this type of analysis (Klug and Alexander[7]).

In laboratory A a photographic internal-standard technique was developed and, later, a Geiger - Müller counter technique that can be used without internal standard. In the course of this work it was found that as the size of quartz diminished below 5μ the X-ray intensity dropped. Etching with hydrofluoric acid restored the original value in the size range 1 to 5μ. These findings have been interpreted as due to an amorphous layer. A number of results obtained with quartz from different sources is shown in Fig. 1, from which it will be apparent that, according to the size range used and whether etched or unetched samples are taken, different standard values for quartz can be obtained in different laboratories, and this is borne out by the results given in Table VI.

Differential thermal analysis—On being cooled down from 600° C, quartz suffers an inversion from the α to the β form in the region of 570° C, this being accompanied by evolution of 1 to 3 calories per g. This effect has been used in differential thermal analysis to measure the amount of quartz in a sample after appropriate calibration (Grimshaw[8]).

The method is not yet as widely used as chemical or X-ray diffraction analysis. With quartz below 3μ Stokes's diameter, similar effects are observed as in X-ray diffraction. Results are low, but after the quartz has been etched with sodium hydroxide they are higher

(Boyer[9]; Dempster and Ritchie[10]). The value of the method may be limited, as recent work on pure quartz from different localities has shown wide variations in peak-area measurements, which ranged from 21 to 57 units (Nagasawa[11]).

Discussion of results

If, as has been shown, the results from all methods of quartz determination vary with the size of the quartz used and with the standard employed in calibrating any particular method, how can comparable results be obtained at all? This was the question the trials were designed to answer.

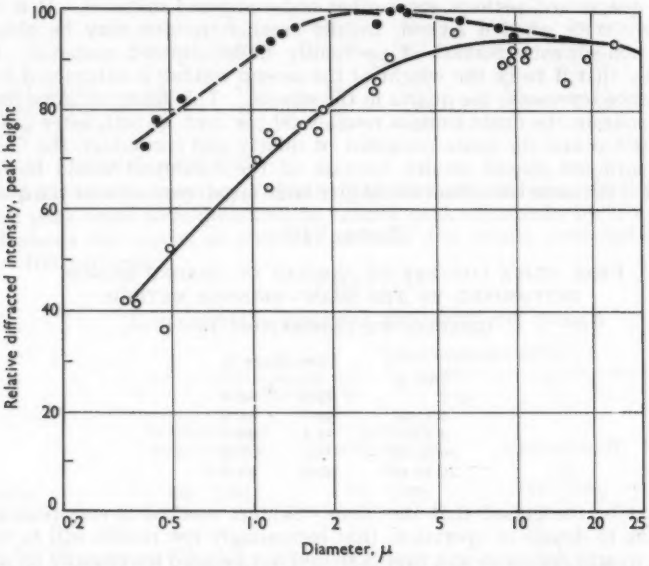

Fig. 1. Variation with particle size of diffracted X-ray intensity from quartz powder: ○ untreated quartz; ● quartz treated with hydrofluoric acid

The first information is provided by examining all results for the quartz sample X1072. Mean values, standard deviation and range of results by various methods are shown in Table IX.

Table IX

Mean results for samples of quartz, X1072

Method	No. of analyses	Mean percentage of quartz	Standard deviation	Range of results, % of quartz
Chemical:				
(*a*) Shaw - Skinner ..	4	89·5	3·0	86 to 92
(*b*) Phosphoric acid ..	5	83·6	7·7	78 to 93
X-ray diffraction	7	84·3	8·9	73 to 98
Differential thermal analysis	4	71·7	8·6	60 to 79

It will be seen that chemical and X-ray methods give similar results, but that adherence to a closely specified chemical method (Shaw - Skinner) halves the standard deviation. All methods give results that are lower than 100 per cent. and the mean values range from 80 to 90 per cent. of quartz, differential thermal analysis tending to give even lower results.

Amongst the other samples there were three mixtures that contained 14, 48·4 and 66·7 per cent., respectively, of the quartz sample X1072. In order to make the analytical results for these samples comparable they have all been corrected by multiplying by $100/F$, where F is the quartz value obtained for X1072 by any particular laboratory and method.

Differential thermal analysis has not been evaluated, as there were too few results. Mean values and standard deviations are shown in Table X. This shows Shaw - Skinner and X-ray results to be about equal in merit. Standard deviations range from 2·5 to 4 irrespective of the actual amount of quartz to be measured. The mean values of quartz X1072 found in the samples approach the theoretical values about equally closely.

TABLE X

MEAN RESULTS OF TRIALS ON THREE MIXTURES, AFTER ALLOWANCE HAS BEEN MADE FOR VARIABILITY OF X1072 RESULTS

Method	No. of analyses	X2479 (14% of X1072) Mean	X2479 (14% of X1072) Standard deviation	X2480 (48·4% of X1072) Mean	X2480 (48·4% of X1072) Standard deviation	X2043 (66·7% of X1072) Mean	X2043 (66·7% of X1072) Standard deviation
Chemical:							
(*a*) Shaw - Skinner ..	4	15·1	3·0	49·5	2·7	67·4	3·8
(*b*) Phosphoric acid ..	5	15·3	3·0	44·7	8·3	73·9	7·7
X-ray diffraction	6	15·3	3·3	47·7	4·1	66·6	3·4

The phosphoric acid methods appear to be less satisfactory. The mean values are too high for sample X2043 and too low for sample X2480, and the standard deviations are more than twice as large as those for the X-ray and Shaw - Skinner methods. Nevertheless, a given laboratory, such as J for instance, may produce excellent results by a phosphoric acid method.

The main conclusion of the trials is that quartz percentages in samples of ash from coal-mine dusts and similar materials of respirable size can be determined by chemical or X-ray diffraction techniques in different laboratories with an accuracy of 7 to 8 units, *i.e.*, 15 $\pm$ 7 per cent., 66 $\pm$ 7 per cent., etc., if the technique has been calibrated against a standard sample of quartz of similar size. Without such calibration the variability between laboratories or methods is much larger.

Although precision is always the ideal aim of an analyst, the purpose of an analysis must not be forgotten. A result, such as that sample A contains 14 to 20 per cent. of quartz, may be far more informative than the apparently more precise statement that sample A contains 57·3 per cent. of SiO_2, which leaves the range of quartz percentage open between the limits of nil and 57.

The choice of method with any given sample depends on laboratory needs and facilities. For a few quartz analyses chemical technique would be the obvious choice; for many analyses X-ray diffractometer methods give far more results per man hour, but the equipment is costly. Differential thermal analysis takes an intermediate position.

The work described in this paper was carried out under the auspices of Panel 2 of the Sub-Committee on Dust of the Medical Research Council's Industrial Pulmonary Diseases Committee, under the chairmanship of Professor E. J. King.

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APPENDIX

DETERMINATION OF QUARTZ BY THE SHAW - SKINNER SEMI-MICRO METHOD

REAGENTS—

Hydrochloric acid, approximately 0·25 N—Dilute 2·5 ml of the concentrated acid to 100 ml with distilled water.

Hydrochloric acid, approximately 0·5 N—Dilute 5 ml of the concentrated acid to 100 ml with distilled water.

Sulphuric acid, approximately 9 N—Dilute 25 ml of the concentrated acid to 100 ml with distilled water.

Sulphuric acid, approximately 3·6 N—Dilute 10 ml of the concentrated acid to 100 ml with distilled water.

Lunge solution—Dissolve 37 g of anhydrous sodium carbonate and 10 g of sodium hydroxide in distilled water and dilute to 1 litre; store the solution in a waxed glass or, preferably, plastic bottle.

Hydrofluoric acid, 40 per cent. w/v.

PROCEDURE—

Dry the sample at 105° C for at least 1 hour, and then heat 0·15 g to boiling with 9 ml of 0·25 *N* hydrochloric acid in a 50-ml platinum basin, covered with a watch-glass, preferably of resistance glass (Pyrex or Hysil). A 3-inch (7·5-cm) porcelain dish may be used, provided that the glaze is in good condition. This treatment is applied to remove carbonates. Set the dish aside to cool, and then decant the liquid down a glass rod into a 15-ml centrifuge tube, which should then be spun in a centrifuge for 15 minutes at 3000 r.p.m. and 14·5 cm radius. Remove the clear supernatant liquid through a tube that has been drawn out to a capillary, the end being turned upwards to avoid disturbing the sediment in the tube. (With care, the liquid may be removed by decanting down a rod, since the sediment is tightly packed.) Wash the solid matter back into the dish with the minimum amount of water, place the dish on a boiling-water bath and evaporate to dryness. When the residue has cooled, add 6 ml of 9 *N* sulphuric acid and heat the uncovered dish until fumes are just visible. This operation should take about 45 minutes and the temperature of the contents of the dish should not exceed 200° C at any time.

After the treatment with sulphuric acid, set the dish aside to cool and then add 30 ml of water; stir the liquid and spin it in a centrifuge as described above; the tubes will have to be filled two or three times. Wash the residue back into the dish, evaporate the liquid to dryness, and repeat the sulphuric acid treatment. When all the liquid has been transferred to the centrifuge tube, rinse the dish and its contents with 5 ml of water, and then spin the solution in a centrifuge. The purpose of this wash is to remove as much as possible of the sulphuric acid and thus reduce the amount of Lunge solution needed in the next stage of the work. After the wash liquid has been spun in a centrifuge and discarded, return the residue to the dish and evaporate the liquid to dryness. To the dry residue add 1 drop of methyl red indicator and then Lunge solution drop by drop until the indicator changes colour. Cover the dish during this operation, since loss can occur owing to effervescence. After neutralisation, add 9 ml of Lunge solution and heat the covered dish until the liquid boils. After the supernatant liquid has cooled, spin it in a centrifuge, discard the clear liquid, wash the residue back into the dish and evaporate to dryness. Add 15 ml of concentrated hydrochloric acid to the covered dish and heat it until its contents have boiled for 5 minutes; when cool, the contents of the dish are diluted with 15 ml of water, which can, with advantage, be used for washing the cover-glass. Decant the liquid, spin in a centrifuge, return the residue to the dish and, after evaporating to dryness, again neutralise the residue with Lunge solution. When neutral, add 4·5 ml of Lunge solution, heat the contents of the dish to boiling and, when cool, decant the liquid, spin in a centrifuge, and wash the residue back into the dish. Evaporate to dryness, and then treat the residue with 6 ml of concentrated hydrochloric acid and boil for 5 minutes. When cold, add 6 ml of water and filter the liquid through a small (7-cm) No. 540 Whatman filter-paper. Wash the residue in the dish twice with 0·5 *N* hydrochloric acid, adding the washings to the paper, and then completely transfer the solid matter in the dish to the paper with the aid of a jet of water, the dish being cleaned finally either with a small rubber-tipped glass rod or, better, with a small piece of cotton-wool twisted round the end of a glass rod. The cotton-wool can be detached easily from the rod and then added to the paper; on ignition it leaves no weighable residue. Wash the paper four times with water and transfer it to a weighed platinum crucible; put the lid on the crucible and heat it in an oven at 105° C until the paper is dry. Place the covered crucible for 10 minutes in the front of a muffle furnace heated to 800° C at its centre; afterwards move it to the centre of the furnace and ignite to constant weight (residue *A*). Finally, treat the ignited residue with 6 drops of 10 per cent. sulphuric acid and 5 ml of 40 per cent. w/v hydrofluoric acid and heat on a boiling-water bath until all the volatile matter has gone. Then heat the crucible on a hot-plate until all the residual

sulphuric acid has been removed, and again ignite the crucible at 800° C to constant weight (residue *B*).

CALCULATION—

The residue (*A*) remaining after the ignition following the last hydrochloric acid treatment consists of the free silica and some undecomposed silicates. The loss in weight caused by the hydrofluoric acid treatment is due to the volatilisation of the free silica and the silica combined in these silicates. The final residue (*B*) therefore consists of the non-siliceous portion, which, after ignition, is largely alumina. The amount of residue *B* multiplied by 2 gives an estimate of the amount of undecomposed silicates. The calculation therefore is as follows—

If *A* is the weight of total residue and *B* is the weight of residue remaining after the hydrofluoric acid treatment, then—

$$\text{free silica present in sample} = \frac{(A - 2B)\,100}{\text{weight of sample}}.$$

The results obtained in the above study suggest that this value should be raised by 10 per cent. when analysing samples of respirable dust, to allow for the solubility of quartz of small size.

In order to obtain reliable results, the conditions must be reproducible, and this can only be done if concentrations of reagents, quantities, times of heating and so on are all strictly controlled.

NOTES ON THE PROCEDURE—

Boiling—The liquid in the dishes is considered to be boiling when drops begin to condense on, and fall from, the watch-glass. This is especially important in the treatments with concentrated hydrochloric acid. Considerable bubbling owing to liberation of carbon dioxide may occur initially, but after about 1 minute the liquid will become still, and proper boiling begins after about another minute. Prolonged boiling should always be avoided.

Interruption—The whole cycle of operations cannot be finished in 1 day; when work has to be stopped, care should be taken that the residues in the dishes are left in the dry state.

Mechanical losses—In such a long series of operations as those described, mechanical losses can be very high. Dishes should be kept covered as much as possible and great care should be taken to see that decantations are done cleanly. Washing the residues back into the dishes from the centrifuge tubes also requires care.

Reagents—Volumes should be measured carefully with a graduated pipette for those less than 10 ml, and a narrow diameter measuring cylinder for the others. The 40 per cent. w/v hydrofluoric acid may be measured in a plastic measuring cylinder. The basins may be heated for the boiling operations on a sand-bath resting on an electric hot-plate, but for evaporation a boiling-water bath should be used. The platinum dishes should be prevented from touching the copper top of the bath by rings of plastic, or other inert material.

REFERENCES

1. Crossmon, G., *Arch. Ind. Hyg.*, 1952, **6**, 416.
2. Thaer, A., *Staub*, 1954, **38**, 555.
3. Durkan, T. M., *J. Ind. Hyg.*, 1946, **28**, 217.
4. Talvitie, N. A., *Anal. Chem.*, 1951, **23**, 623.
5. Trostel, L. J., and Wynne, D. J., *J. Amer. Ceram. Soc.*, 1940, **23**, 18.
6. Shaw, A., *Analyst*, 1934, **59**, 446.
7. Klug, H. P., and Alexander, L. E., "X-ray Diffraction Procedures," Chapman & Hall Ltd., London, 1954.
8. Grimshaw, R. W., *Clay. Min. Bull.*, 1953, **2**, No. 9, 2.
9. Boyer, A. F., *J. Chim. Phys.*, 1953, **50**, 60.
10. Dempster, P. B., and Ritchie, P. D., *Nature*, 1952, **169**, 538.
11. Nagasawa, K., *J. Earth. Sci., Nagoya Univ.*, 1953, **1**, 156.

SAFETY IN MINES RESEARCH ESTABLISHMENT
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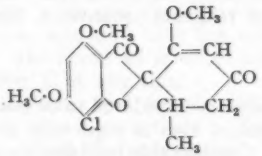
Determination of Griseofulvin in Fermentation Samples

Part I. Spectrophotometric Assay

By G. C. ASHTON and A. P. BROWN

A physico-chemical procedure for the determination of griseofulvin in fermentation broth samples is described. The method involves solvent extraction of the whole-broth sample with butyl acetate and measurement of the ultra-violet spectrophotometric absorption of the extract. A correction procedure is described (in the Appendix to Part I) that allows mathematically for irrelevant ultra-violet absorption in the extract.

Griseofulvin was first isolated by Oxford, Raistrick and Simonart[1] from *Penicillium griseofulvum* Dierckx grown in surface culture. The structural formula of griseofulvin determined by Grove, MacMillan, Mulholland and Rogers[2] is shown below—



Brian, Curtis and Hemming[3,4] later isolated the same substance from several different *Penicillium* spp. More recently it has been found possible to grow griseofulvin-producing penicillia under deep fermentation conditions, with considerably increased yields.

The development of such fermentation procedures, together with the increasing interest in the agricultural application of griseofulvin as a systemic fungicide, has made it necessary to have suitable assay procedures for control and standardisation. Brian *et al.*[3] have described a biological assay based on the curling of *Botrytis allii* hyphae in the presence of griseofulvin. However, this assay is rather imprecise in our experience and difficult to apply routinely. This paper describes the development of a spectrophotometric assay, suitable for control purposes, based on the absorption spectrum of griseofulvin.

Experimental

Extinction values of griseofulvin—

The ultra-violet absorption spectrum of griseofulvin in various solvents is shown in Fig. 1. Grove *et al.*[5] quote a logϵ of 4·34 at 291 mμ in methanol, corresponding to an $E^{1\%}_{1cm}$ at that wavelength of 621 (molecular weight of griseofulvin is 352·5). We had obtained values higher than this, and a quantity of very pure griseofulvin was therefore prepared to establish the extinction values in various solvents.

Griseofulvin with an extinction value of 670 on our instrument at 295·7 mμ in 0·2 per cent. aqueous dimethylformamide solution was dissolved in benzene at room temperature until a saturated solution was obtained. The benzene was progressively evaporated at room temperature in a stream of dry air. Colourless crystals of griseofulvin were deposited on the walls of the container: they were washed with benzene, the solvent was evaporated under reduced pressure, and the crystals were dried at 100° C for 2 hours. The extinction value of this material at 295·7 mμ in 0·2 per cent. aqueous dimethylformamide was 688 on our instrument.

A quantity of this material was sublimed at 210° C at atmospheric pressure. The extinction value of the sublimate at 295·7 mμ was also 688 on our instrument. Several preparations from the original starting material gave the same values.

The instrument for determining these extinction values was a much-used Unicam SP500. Although assays on this instrument could be compared with the extinction values established

on the same instrument, for inter-laboratory comparison it was desirable to obtain an absolute value for the extinction of pure griseofulvin in one solvent. The instrument was therefore standardised with 0·005 per cent. of potassium dichromate in 0·1 *N* sulphuric acid as described by Cama, Collins and Morton.[6]

The corrected value for the extinction value of griseofulvin in 0·2 per cent. aqueous dimethylformamide is 705 at 295·7 mμ. This value was confirmed independently on a sample of sublimed griseofulvin on two Hilger Uvispek absorptiometers in good condition, values of 702 and 705 being obtained at 295·5 to 296·0 mμ on freshly prepared solutions.

The extinction value of pure griseofulvin in butyl acetate was found to be 705 at 289·25 mμ. The extinction value in methanol is approximately 680 at 291·5 mμ, but in our experience griseofulvin is not stable in this solvent.

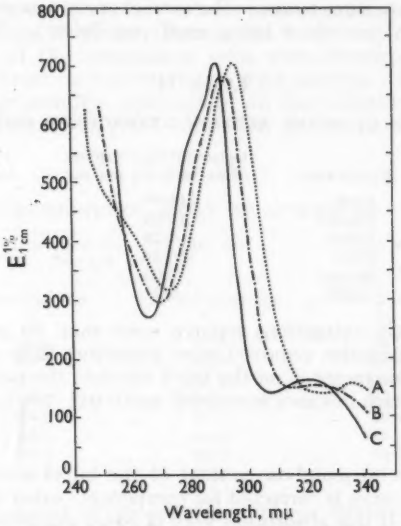


Fig. 1. Ultra-violet absorption of griseofulvin in different solvents. Curve A, in 0·2 per cent. aqueous dimethylformamide; curve B, in methanol; curve C, in butyl acetate

SOLVENT EXTRACTION OF FERMENTATION MATERIAL—

At least 80 per cent. of the griseofulvin in fermentation broth samples is in the mycelium, from which it is necessary to extract it; for doing this several solvents were tried in preliminary experiments. Aliquots of whole broth were extracted with four volumes of the solvent three times, the extracts then being pooled and diluted to 100 ml. One-ml quantities of the extracts were evaporated to dryness in a stream of dry air and the residues dissolved in 5 ml of methanol. The amount of griseofulvin in the methanol extracts was determined. The results are shown in Table I.

TABLE I

GRISEOFULVIN EXTRACTED BY DIFFERENT SOLVENTS

Solvent	Griseofulvin in methanol extract, μg per ml
Butyl acetate	6·3
Methyl Cellosolve	7·3
Dimethylformamide	6·8
Methanol	7·1
*cyclo*Hexane	0·8
Ether	6·2
Chloroform	6·4
Benzene	5·1

The results in Table I are not corrected for irrelevant absorption (see below) and except for the *cyclo*hexane extract the results are not essentially different. Of the solvents examined, butyl acetate proved to be most convenient in practice, being relatively immiscible with whole broth and transmitting in ultra-violet light down to 270 mμ. Most of the other solvents gave emulsions on shaking with whole broth.

Various extraction procedures with butyl acetate and whole broth were tried, including mixing in a Waring Blendor, manual shaking in a separating funnel and manual and mechanical shaking in a boiling tube. Repetitive extraction of a small volume (2 to 10 ml) of whole broth in a boiling tube with four volumes of butyl acetate proved satisfactory. Altering the pH of the broth had no effect on the amount of griseofulvin extracted.

The number of extractions required to effect complete removal of griseofulvin was determined. Two broth samples were extracted by shaking one volume of each sample with four volumes of butyl acetate three times. The extracts were assayed spectrophotometrically, the seven-point correction procedure being used (see below). The results are shown in Table II.

TABLE II

NUMBER OF BUTYL ACETATE EXTRACTIONS REQUIRED

Sample	Extraction	Griseofulvin content of extract, μg per ml	Percentage of total extracted
26Q1	First	214·50	94·6
26Q1	Second	10·95	4·8
26Q1	Third	1·26	0·6
26Q2	First	184·86	95·2
26Q2	Second	8·65	4·4
26Q2	Third	0·71	0·4

It is apparent that two extractions remove more than 99 per cent. of the available griseofulvin. The third extraction removed more impurities than griseofulvin, as evidenced by the large correction value required on the third extract; the non-corrected and corrected absorbances at the peak in each instance were 0·560 and 0·041 (26Q1), 0·347 and 0·049 (26Q2).

CORRECTION PROCEDURE—

A true estimate of the griseofulvin content of the butyl acetate extract can only be obtained if the absorption curve is corrected for components other than griseofulvin, that is, for irrelevant absorption. If this absorption were of equal magnitude over a suitable range,

TABLE III

REPRODUCIBILITY OF SPECTROPHOTOMETRIC ASSAY AS APPLIED TO FERMENTATION SAMPLES

Sample	Diluted extract assay		
	a, μg per ml	*b*, μg per ml	*c*, μg per ml
R28/R1	9·17	9·20	9·03
R27/R2	7·73	7·42	7·46
PC1	8·27	7·96	8·34
PC2	8·60	8·56	8·43
IDI	8·84	8·98	8·70
Q36/Q1	5·17	5·25	5·33
Q36/Q2	8·00	7·92	8·00
Q38/Q1	8·79	8·71	9·50
Q38/Q4	6·13	6·25	6·17
Q38/Q2	6·25	6·18	6·22
R39/R2	6·65	7·02	7·16

say 289 to 325 mμ, the griseofulvin content could be calculated by subtracting the 322·5-mμ peak value from the 289-mμ peak value and relating this difference to the corresponding difference for pure griseofulvin. In practice, a useful estimate of the approximate potency of a broth sample can be obtained in this way when previous experience has shown that the medium and organism employed produce ultra-violet absorbing components that are mainly griseofulvin.

A correction procedure described by Morton and Stubbs[7] and widely applied in the spectrophotometric determination of vitamin A was examined in relation to the griseofulvin determination. For reasons discussed in the Appendix to Part I, this three-point correction procedure was not considered suitable, and an alternative procedure based on readings at seven wave-lengths was developed.

PRECISION OF SPECTROPHOTOMETRIC ASSAY—

Several broth samples were assayed in triplicate by the method described, with correction for irrelevant absorption by the seven-point procedure. The results are shown in Table III.

The standard deviation of a single determination on these fermentation samples averaged ± 2·4 per cent.

The purity of a number of broth solids was determined spectrophotometrically under routine conditions. Griseofulvin is not very soluble in the common organic solvents, whereas it is readily soluble and stable in dimethylformamide.[8]

Approximately 50 mg of the griseofulvin solid were dissolved in 25 ml of dimethylformamide, in duplicate. From each solution two 5-ml portions were each diluted to 1 litre with butyl acetate. The griseofulvin contents of all four dilutions were then determined spectrophotometrically by the seven-point correction procedure. Some results are shown in Table IV.

TABLE IV

PRECISION OF SPECTROPHOTOMETRIC ASSAY AS APPLIED TO SOLID PREPARATIONS

Sample	Purity after first weighing and primary dilution		Purity after second weighing and primary dilution	
	First dilution, %	Second dilution, %	First dilution, %	Second dilution, %
DB84	98·0	98·5	96·0	96·5
DB117	95·9	95·8	97·4	96·0
DB118	99·8	101·2	98·7	99·9
DB126	86·2	86·1	87·2	87·1
DC300	87·2	88·4	88·4	89·3
DC450	86·0	84·3	85·7	86·5
DD83	98·2	98·0	96·6	96·4
DD134	95·7	96·0	94·4	94·0

A single determination based on one weighing and one dilution has a standard deviation averaging ± 1·6 per cent., this error being composed partly of errors due to weighings and primary and secondary dilutions and partly of the error of the spectrophotometric measurements.

METHOD

PROCEDURE—

Measure 5 ml of well mixed whole-broth sample into a 6-inch × 1-inch boiling tube. Add 20 ml of reagent grade butyl acetate, stopper the tube and shake hard for 2 minutes. Decant as much of the butyl acetate extract as possible; re-extract with a further 20-ml quantity of butyl acetate. Combine the two extracts and dilute to 100 ml with butyl acetate. Filter a quantity of the diluted extract through a No. 54 Whatman filter-paper.

Dilute an aliquot of the filtered extract with butyl acetate to give a solution containing between 5 and 15 μg of griseofulvin per ml.

Measure the ultra-violet absorption of this dilute solution against butyl acetate at 288, 290, 292, 294, 296, 298 and 300 mμ in 1-cm silica cells. It is not necessary to know the blank values of the cells, but they should be uniform or linear over the wavelength range employed.

CALCULATION—

The calculation of the result from the spectrophotometric measurements is based on quadratic curvature corrected coefficients. The calculation of these coefficients is described in the Appendix to Part I.

The absorbance at each of the seven wavelengths is multiplied by the coefficient for that wavelength, the products are summed with due regard to algebraic sign, and the sum of the products is divided by the divisor. The result is in terms of μg of griseofulvin per ml

in the solution measured on the spectrophotometer. The result is then multiplied by 20 to allow for the broth volume (5 ml) and the extract volume (100 ml) and by any dilution factors involved in preparing the solution for spectrophotometry. An example to illustrate the calculation is as follows—

Column I wavelength	Column II absorbance	Column III coefficient	Column IV coefficient × absorbance
288	0·562	− 10·738	− 6·034756
290	0·568	+ 10·872	+ 6·175296
292	0·494	+ 11·478	+ 5·670132
294	0·372	− 1·612	− 0·599664
296	0·253	− 11·682	− 2·955546
298	0·176	− 7·644	− 1·345344
300	0·134	+ 9·326	+ 1·249684
		Total	2·159802

Divisor = 0·2585.

Therefore, concentration of measured solution $= \frac{2{\cdot}159802}{0{\cdot}2585} = 8{\cdot}36\ \mu g$ per ml.

Broth titre $= 8{\cdot}36 \times \frac{100}{5} \times$ butyl acetate dilution factor.

Results

Some results obtained by the spectrophotometric assay are discussed in Part II.

The authors are indebted to Miss I. Thompson and Mrs. E. Hockley for technical assistance, and to Mr. W. H. C. Shaw for measurement of some extinction values on samples of pure griseofulvin.

References

1. Oxford, A. E., Raistrick, H., and Simonart, P., *Biochem. J.*, 1939, **33**, 240.
2. Grove, J. F., MacMillan, J., Mulholland, T. P. C., and Rogers, M. A. T., *J. Chem. Soc.*, 1952, 3977.
3. Brian, P. W., Curtis, P. J., and Hemming, H. G., *Trans. Brit. Mycol. Soc.*, 1946, **29**, 173.
4. ——, ——, ——, *Ibid.*, 1949, **32**, 30.
5. Grove, J. F., MacMillan, J., Mulholland, T. P. C., and Rogers, M. A. T., *J. Chem. Soc.*, 1952, 3949.
6. Cama, H. R., Collins, F. D., and Morton, R. A., *Biochem. J.*, 1952, **50**, 50.
7. Morton, R. A., and Stubbs, A. L., *Analyst*, 1946, **71**, 348.
8. Ashton, G. C., and Rhodes, A., *Chem. & Ind.*, 1955, 1183.

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Determination of Griseofulvin in Fermentation Samples

Appendix to Part I: Seven-point Correction Procedure

By G. C. ASHTON and J. P. R. TOOTILL

In the spectrophotometric determination of griseofulvin in fermentation samples, it is necessary to correct the ultra-violet absorption values of butyl acetate extracts for irrelevant absorption, that is, for absorption not due to griseofulvin itself. This problem is a general one with ultra-violet spectra of impure preparations or extracts, and a commonly applied correction procedure is that due to Morton and Stubbs[1] for vitamin A in fish oils. Their three-point correction assumes that three wavelengths can be found at which the irrelevant absorption can be joined by a straight line. Such an approach is empirical and the choice of suitable wavelengths difficult.[2]

In our opinion a better method would be to apply a correction procedure that is determined by the shape of the irrelevant-absorption curve and corrects for it. This procedure can only be used when the form of the irrelevant absorption is less complex than that of the substance being assayed; fortunately, with griseofulvin extracts we have found that the irrelevant absorption with material from the medium and strain employed gives a quadratic curve.

Determination of the shape of the irrelevant-absorption curve—

Preliminary experiments were carried out to establish the reference curve obtained with pure griseofulvin in butyl acetate at 2-mμ intervals with a high degree of precision. On any one replicated experiment a very small error was obtained, but it was not found possible to reproduce the reference absorption curve subsequently within this error. It seemed probable that this difficulty was due to slight differences in wavelength setting from day to day, coupled with the very steep slope of both approaches to the griseofulvin peak at 289·25 mμ. Consequently, in the mathematical analysis of broth-extract absorbances in butyl acetate, it was not possible to determine the shape of the irrelevant-absorption curve by comparison with a previously established reference curve.

When standard solution and butyl acetate extract were read side by side, each at the same wavelength scale settings, this difficulty was overcome and it was possible to analyse the results mathematically. Typical results are shown in Table I.

Table I

Analysis of variance of results shown graphically (Fig. 2)

Source of variation	Degrees of freedom	Mean square
Uncorrected griseofulvin estimate	1	2·764,139,665
Correction for uniform absorption	1	0·007,478,159
Additional correction for linear absorption ..	1	0·000,090,070
Additional correction for quadratic absorption ..	1	0·000,207,345
Additional correction for cubic absorption ..	1	0·000,001,089
Residual absorption	4	0·000,000,918

The standard error per observation is known to be approximately 0·001 absorbance unit; hence neither the cubic correction nor the residual error indicate any significant absorption of higher complexity than quadratic.

The nature of the irrelevant absorption in this particular sample is shown in Fig. 2. Although in such precise work as that in the experiment summarised above the wavelength setting was a critical factor, it was found experimentally that good reproducibility could be obtained in practice by establishing a set of coefficients describing the standard curve and using these to correct for irrelevant absorption in broth extracts (Table III, see below).

BASIS OF QUADRATIC CORRECTION COEFFICIENTS AND CHOICE OF WAVELENGTHS—

Irrelevant absorption can be corrected for by developing a set of coefficients related to the absolute values of the reference ultra-violet absorption curve but independent of uniform, linear or quadratic absorption. The derivation of such coefficients is shown below by reference to a set of experimental results.

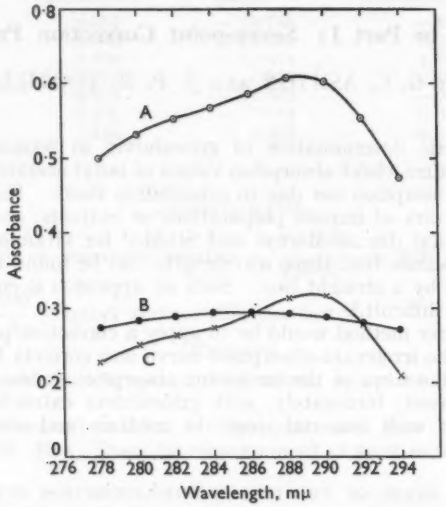

Fig. 2. Irrelevant absorption in butyl acetate extracts containing griseofulvin. Curve A, total absorption of butyl acetate extract; curve B, irrelevant absorption in extract; curve C, curve due to griseofulvin in extract

The number of wavelength readings, the spacing of the readings and the position of the chosen wavelengths in relation to the reference curve require consideration. Although quadratic irrelevant absorption can be detected and allowed for with four points, a rather imprecise result would be obtained unless the readings were replicated. A larger number of wavelength readings is therefore desirable and, although any number could be used, seven readings were chosen. A wavelength spacing of 2 mμ seemed a suitable value with our instrument (Unicam SP500 quartz spectrophotometer).

The choice of wavelengths was decided by establishing values for a standard solution at 278 to 302 mμ in steps of 2 mμ. Successive groups of seven wavelength readings were compared with the aid of orthogonal polynomials and that portion of the reference curve with the highest cubic component was chosen. The seven wavelengths thus found were 288 to 300 mμ in steps of 2 mμ.

CALCULATION OF QUADRATIC CORRECTION COEFFICIENTS—

The mechanics of calculating the coefficients is best described by reference to an example (Table II).

The reference solutions are prepared in triplicate by dissolving approximately 10 mg of pure griseofulvin in 5 ml of dimethylformamide and diluting to 1 litre with butyl acetate.

REPRODUCIBILITY OF RESULTS WITH CORRECTION COEFFICIENTS

As pointed out above, the reference absorption curve is not reproducible from day to day within the small limits of error of a single reading on the instrument. However, in practice it is not convenient to read a standard and unknown side by side at seven wavelengths and calculate an individual set of coefficients for such readings. Thus a compromise is necessary, resulting in some loss in accuracy but a great saving in computational labour. The effect of computing the griseofulvin concentration of four butyl acetate extracts measured individually against four separately determined sets of correction coefficients is shown in Table III.

TABLE II

CALCULATION OF COEFFICIENTS CORRECTING FOR QUADRATIC CURVATURE

Wavelength, mμ	Standard solutions absorbances = S: 9·811 μg per ml	10·031 μg per ml	10·080 μg per ml	I Sum of absorbances of standard solutions ΣS	II Linear coefficients X_1	III I × II $X_1\Sigma S$	IV Curvature* coefficients X_2	V I × IV $X_2\Sigma S$	VI Correction coefficients (formula below)
288	0·656	0·668	0·672	1·996	− 3	− 5·988	+ 5	+ 9·980	− 10·738
290	0·663	0·673	0·679	2·015	− 2	− 4·030	0	—	+ 10·872
292	0·580	0·590	0·592	1·762	− 1	− 1·762	− 3	− 5·286	+ 11·478
294	0·437	0·442	0·445	1·324	0	—	− 4	− 5·296	− 1·612
296	0·298	0·300	0·302	0·900	+ 1	+ 0·900	− 3	− 2·700	− 11·682
298	0·206	0·207	0·209	0·622	+ 2	+ 1·244	0	—	− 7·644
300	0·156	0·159	0·161	0·476	+ 3	+ 1·428	+ 5	+ 2·380	+ 9·326
Sums of columns I to VI				(ΣS) = 9·095 (ΣS^2) = 14·325321	Zero	($X_1\Sigma S$) = − 8·208	Zero	($X_2\Sigma S$) = − 0·922	Zero

NOTES—

1. The values in column VI are computed at each wavelength from the formula—
$$84\Sigma S - 12(\Sigma S) - 3X_1(X_1\Sigma S) - X_2(X_2\Sigma S).$$
2. The divisor is computed from the formula: $\dfrac{84(\Sigma S^2) - 12(\Sigma S)^2 - 3(X_1\Sigma S)^2 - (X_2\Sigma S)^3}{\text{sum of solution concentrations in } \mu\text{g per ml}} = 0{\cdot}2585,$
where (ΣS^2) is the sum of the squares of the values in column I.

CHECKS—

(*a*) The correction coefficients (column VI) should sum to zero.
(*b*) Multiply the values in column I by the appropriate coefficient in column VI, sum the products algebraically, and divide by the calculated divisor. The result should be the sum of the concentrations of the three standard solutions in μg per ml.

* From Fisher and Yates.[3]

TABLE III

REPRODUCIBILITY OF RESULTS WITH SEPARATELY DETERMINED CORRECTION COEFFICIENTS

Butyl acetate extract	Correction coefficients: *a*	*b*	*c*	*d*
Q1 ..	8·36	8·30	8·59	8·38
Q2	7·95	7·91	8·19	7·98
F1	8·41	8·37	8·66	8·44
F2	8·16	8·12	8·40	8·19

Figures represent griseofulvin content of butyl acetate extracts in μg per ml.

It can be seen that the error involved by this compromise procedure is of the same order as that between replicate broth extracts (Part I, Table III, p. 222).

DISCUSSION

Owing to the difficulty of reproducing the reference curve already mentioned, it has not been possible to ascertain exactly the optimal portion of the curve to use. It may be that more exhaustive examination would indicate a different choice of wavelengths.

A further discussion of the theoretical basis for this correction procedure, and of the precision of this correction, is given in the paper by Ashton and Tootill[4] in this issue.

REFERENCES

1. Morton, R. A., and Stubbs, A. L., *Analyst*, **1946**, **71**, 348.
2. Gridgeman, N. T., *Ibid.*, 1951, **76**, 449.
3. Fisher, R. A., and Yates, F., "Statistical Tables for Biological, Agricultural and Medical Research," Fourth Edition, Oliver & Boyd, Edinburgh, 1953, p. 80 *et seq.*
4. Ashton, G. C., and Tootill, J. P. R., *Ibid.*, 1956, **81**, 232.

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Determination of Griseofulvin in Fermentation Samples

Part II: Isotope-dilution Assay

By G. C. ASHTON

To confirm the results of the spectrophotometric assay (Part I), and also to provide an alternative procedure for the determination of griseofulvin, an isotope-dilution assay based on chlorine-36 griseofulvin has been developed. Results obtained by this procedure are compared with those by the spectrophotometric method.

The choice of tracer for the preparation of the labelled griseofulvin lay between carbon-14 and chlorine-36. It was found that radioactive chlorine was readily incorporated into the griseofulvin molecule in good yield and so this tracer was chosen. It has the further advantage that material so labelled can be distinguished from dechlorogriseofulvin,[1,2] which may be present, by alumina chromatography.[2]

Preparation of chlorine-36 griseofulvin "label"—

The chlorine-36 griseofulvin was prepared by submerged fermentation with the strain and medium described elsewhere[3]; this strain does not produce dechlorogriseofulvin in submerged culture under a variety of conditions. The medium contained $K^{36}Cl$.*

The harvested broth volume was measured (200 ml) and the whole broth extracted four times with an equal volume of ether. The pooled ether extracts were filtered through a Whatman No. 54 filter-paper. Dimethylformamide (20 ml) was added to the filtered ether extract and the ether was distilled from a water bath or low-temperature hot-plate. The dimethylformamide residue was treated with 160 ml of water and the precipitated griseofulvin filtered off. The precipitate was dissolved in dimethylformamide and a sufficient quantity of carrier (99·9 per cent. pure griseofulvin) added to bring the activity of the final isolated material to approximately 0·1 μC per g. The volume of dimethylformamide used was sufficient to give a 5 per cent. concentration of griseofulvin; eight volumes of water were then added to reprecipitate the "diluted" griseofulvin. The precipitate was filtered off, dried and dissolved in the minimum quantity of warm benzene. The benzene solution was filtered and evaporated at room temperature to a small volume in a stream of dry air. Griseofulvin crystallised as the volume decreased and was successively removed from the mother liquors. The crystalline material was freed from benzene by heating at 100° C to constant weight. The final product was 99·8 per cent. pure by seven-point spectrophotometric assay, with a m.p. 221° to 222° C. It was free from dechlorogriseofulvin when tested by MacMillan's nitric acid test.[2] About 10 g of suitably diluted material were obtained from 10 μC of chlorine-36 as $H^{36}Cl$.

The specific activity of the non-diluted griseofulvin was found to be 5·175 and 5·023 μC per g in two series of fermentations; 45·7 and 44·3 per cent. of the respective preparations were labelled with chlorine-36. The percentage of added chlorine-36 taken up from the medium into the griseofulvin produced was 19·3 per cent. in one preparation and 32·6 per cent. in another.

Experimental

The experimental work associated with an isotope-dilution assay is essentially concerned with the development of a small-scale extraction process to yield either the diluted label in pure form or else a pure degradation product containing the isotopically labelled moiety. In this instance it was found possible to isolate griseofulvin free from contaminants and in a form suitable for counting, so that recourse to the preparation of a derivative was not necessary.

* The chlorine-36 was purchased from the Radiochemical Centre, Amersham, Bucks., in the form of 2*N* hydrochloric acid, 1·0 ml of which contained 8 μC of chlorine-36.

EXTRACTION PROCEDURE—

The experimental procedure envisaged was to extract a volume of whole broth containing 100 to 150 mg of griseofulvin with a suitable solvent, add 50 mg of labelled material, distil off the solvent to a low volume, precipitate the diluted "label" and purify on an alumina column.

Butyl acetate was examined, as it had proved suitable for the spectrophotometric assay. However, it was found that on vacuum-distillation griseofulvin was slightly degraded in this solvent in the presence of dimethylformamide. Of other solvents examined ether was found to be satisfactory, no destruction of griseofulvin occurring on boiling it off. The extraction efficiency from whole broth into an equal volume of ether was found to be 70 per cent., indicating that four extractions with an equal volume of ether would remove over 99 per cent. of the griseofulvin present.

It was convenient to boil off the ether in the presence of a small volume (2 to 5 ml) of dimethylformamide, as the griseofulvin remained in this solvent and could then be precipitated with at least four volumes of water, leaving only 2 to 4 per cent. of the griseofulvin in solution.

It is, of course, essential that the isolated griseofulvin sample for counting should be free from dechlorogriseofulvin. In the absence of this latter substance in the fermentation (which can be checked by isolating a small sample and testing by the nitric acid test[2]) the chromatographic procedure described by Grove *et al.*[4] is excellent for purifying fermentation extracts. If dechlorogriseofulvin occurs in the fermentation sample to be assayed, the chromatographic separation described by MacMillan[2] must be applied. In the method described below it is assumed that dechlorogriseofulvin is absent, and the alumina chromatographic procedure employed is essentially that of Grove *et al.*[4] It was necessary to use an all-glass column to prevent the isolated material from becoming discoloured.

The solvent was readily removed in a stream of dry air at room temperature. The final traces of solvent could be removed by heating the solid at 100° C. Destruction of the griseofulvin did not take place at this temperature, neither was the radioactivity of the solid affected by this treatment.

The purity of the isolated solid may be checked by the seven-point spectrophotometric assay. A sample of "label" was taken through the extraction process; its radioactivity was unchanged by the manipulations involved.

COUNTING PROCEDURE—

Chlorine-36 is a medium-energy β-particle emitter, and as such may be counted in a liquid or end-window counter. We prefer to use an end-window counter, as it gives, in our experience, better replication of counts and a lower specific activity label may be used. With a 1 sq.cm planchette and a G.E.C. type EHM2S Geiger - Müller tube, 30 mg of label of specific activity 0·1 μC per g gave a count of about 1300 to 1400 counts per minute. The weight of griseofulvin on the planchette should be $30 \pm 0{\cdot}1$ mg, as this weight is not on the "infinite thickness" part of the response curve. It was found that replicate platings gave counts agreeing within 2 per cent. or less by this procedure.

ACCURACY AND PRECISION OF THE ISOTOPE ASSAY—

The determination of a substance by dilution with a radioactive one is a fundamentally accurate procedure provided due attention is paid to the purity of the label and isolate. However, the precision of the measurement of the isotope dilution is dependent on the ratio of sample to label, on the relative times allocated to the label, sample and background counts and on the total count time. The conditions for obtaining the best precision have been discussed elsewhere.[5,6]

METHOD

PROCEDURE—

Extract a volume of whole broth containing 100 to 150 mg of griseofulvin four times with an equal volume of ether. The ether extraction must be quantitative; the isotope dilution is based on the amount of griseofulvin extracted.

Pool the ether extracts and filter through a Whatman No. 54 filter-paper. Dissolve approximately 50 mg of griseofulvin label in 5 ml of dimethylformamide in a 250-ml beaker. Put 100 ml of the filtered ether extract into the beaker and distil off the ether on a water bath, replenishing the beaker contents as necessary until all the ether has been boiled off.

Cool the dimethylformamide residue and add 8 to 10 volumes of water. Set the precipitate aside overnight, and then filter through a small Buchner funnel containing a Whatman No. 54 filter-paper. Dry the precipitate at 100° C and dissolve the dried precipitate in 3 ml of benzene by warming in a water bath.

In the absence of dechlorogriseofulvin (see p. 229) prepare an alumina column 1 cm in diameter and 10 to 12 cm high by pouring in a slurry of activated alumina in a mixture containing 99 per cent. of benzene and 1 per cent. of ethanol. The activated alumina is prepared by washing Peter Spence grade 0 alumina to pH 4·0 to 4·5 with sulphuric acid, washing with water until the washings are free of sulphate and heating the filtered acid-washed alumina at 150° C overnight. Drain the column to $\frac{1}{4}$ inch above the surface and apply the benzene solution of griseofulvin. Develop the column with a mixture containing 99 per cent. of benzene and 1 per cent. of ethanol until the griseofulvin, which fluoresces bright blue in ultra-violet light, is eluted. The griseofulvin-containing eluate should then be evaporated in a stream of dry air at room temperature.

Dissolve the benzene residue in 2 ml of dimethylformamide. Filter through a small Hirsch filter with a Whatman No. 54 filter-paper into a 50-ml centrifuge tube. Add 25 to 30 ml of water, centrifuge the contents and decant. Dry the precipitate at 150° C, cool, and wash twice with a small quantity of dry ether to remove any residual dimethylformamide.

RADIOACTIVITY DETERMINATION—

The dried precipitate should be colourless; the purity, which should be determined by the spectrophotometric procedure (Part I), should not be less than 98 per cent. The material should be free from dechlorogriseofulvin by the nitric acid test.[2]

Grind the dried residue in a small mortar to a fine powder, and place 30 $\pm$ 0·1 mg on a 1 sq. cm planchette; level the surface with a suitably sized steel mandrel. Determine the radioactivity of the prepared planchette by means of a lead castle and suitable end-window counter (*e.g.*, a G.E.C. type EHM2S).

At the same time prepare a similarly weighted planchette of the griseofulvin-label, and determine the radioactivity of this planchette also.

CALCULATION—

The griseofulvin content of the whole broth sample is given by—

$$\left(\frac{C_L \times W_T}{C_M} - W_T\right) \times \frac{1000}{V} = \mu g \text{ of griseofulvin per ml,}$$

where C_L = the count of the label (counts per minute), less the background count,
C_M = the count of the isolated griseofulvin sample, less the background count,
W_T = the weight of label used in mg, and
V = the volume of whole broth in ml.

The precision of a single isotope assay of this type can be calculated if necessary.[6]

RESULTS

COMPARISON OF ISOTOPE-DILUTION AND SPECTROPHOTOMETRIC ASSAYS—

The griseofulvin content of a number of whole-broth samples was determined in duplicate by the isotope assay and compared with results obtained spectrophotometrically, see Table I.

The values in Table I are consistent within the limits of error of both assays.

Because most of the griseofulvin of whole broth is located in the mycelium, it is not possible to carry out "recovery assays." Thus the validity of either assay method lies in the consistency of results obtained by the two methods.

Several comparisons between the spectrophotometric assay and the bio-assay of Brian *et al.*[7] were made. Thus aliquots of the butyl acetate extracts from the spectrophotometric assay found by this assay to contain 210 μg were evaporated at room temperature. The residues were dissolved in 1 ml of dimethylformamide and diluted with 20 ml of water. The resulting solutions were bio-assayed against a standard containing 10 μg of pure griseofulvin per ml. In all tests carried out the butyl acetate extracts and the standard gave curling at the same dilution within the rather wide limits of error of the method.

DISCUSSION

For routine purposes the spectrophotometric assay with the seven-point correction procedure is suitable. The major object of developing an isotope-dilution procedure was

TABLE I

ISOTOPE-DILUTION AND SPECTROPHOTOMETRIC ASSAY RESULTS

Sample	Isotope-dilution assay: griseofulvin in sample taken		Spectrophotometric assay: griseofulvin in corresponding volume of sample, mg
	Assay 1, mg	Assay 2, mg	
36Q1/9	59·0	51·0	56·0
36Q1/11	37·1	40·0	44·8
36Q2/11	79·0	79·0	82·5
36Q1/14	34·0	35·5	39·5
36Q2/14	81·0	85·5	86·5
36Q1/16	80·3	81·7	78·9
38Q4/15	65·7	67·1	66·6
39R2/11	96·0	88·5	83·5
38Q2/18	76·0	77·5	74·5
38Q1/20	108·0	96·5	109·2
39R2/14	108·0	118·3	109·5

to verify results got by the spectrophotometric assay. Thus whenever it is necessary to confirm a spectrophotometric assay result, for example, in work involving a new medium or a new strain, the isotope assay is valuable.

Various other assay procedures have been examined, including a polarimetric assay based on the high positive dextrorotation of griseofulvin, and several colorimetric procedures. Of the colorimetric procedures the rather non-specific reaction with sulphuric acid[4] proved unsuitable, owing to the presence of interfering substances in all extracts except those in *cyclo*hexane (Part I, Table I, p. 221).

I am indebted to Miss I. Thompson for technical assistance and to Mr. R. McWilliam for carrying out the chlorine-36 griseofulvin fermentations.

REFERENCES

1. MacMillan, J., *Chem. & Ind.*, 1951, 719.
2. ——, *J. Chem. Soc.*, 1953, 1697.
3. British Patent Application No. 9012/55.
4. Grove, J. F., MacMillan, J., Mulholland, T. P. C., and Rogers, M. A. T., *J. Chem. Soc.*, 1952, 3949.
5. Rodin, N. S., *Nucleonics*, 1947, **1**, 48.
6. Ashton, G. C., and Foster, M. C., *Analyst*, 1955, **80**, 123.
7. Brian, P. W., Curtis, P. J., and Hemming, H. G., *Trans. Brit. Mycol. Soc.*, 1946, **29**, 173.

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A Method of Correcting for Irrelevant Absorption in Ultra-violet Spectrophotometric Analysis

By G. C. ASHTON and J. P. R. TOOTILL

A method of correcting for impurities having quadratic absorption spectra in samples for ultra-violet spectrophotometric analysis, described previously as part of a spectrophotometric assay for griseofulvin, has been applied to experimental mixtures of griseofulvin and materials showing various types of irrelevant absorption. The previously described correction procedure is shown to be highly satisfactory for such irrelevant absorption.

The extension of the correction procedure to other types of irrelevant absorption and to mixtures of substances absorbing in the ultra-violet region is discussed. The effect of this type of correction procedure on the precision of the estimate and a method for determining the degree of curvature of the irrelevant-absorption spectrum are described in Appendixes.

The determination of a substance by measuring its absorption in the ultra-violet region is much-practised. When the spectrum shows a convenient peak or peaks, *i.e.*, regions of sharply defined maximum absorption, measurement of the extinction value at the point of maximum absorption will provide a precise and accurate measure of the substance, providing it is pure. However, it is unusual to deal with pure substances in analysis; more often than not use of an ultra-violet spectrophotometric method is difficult or impossible, because of the presence of irrelevant absorption in the same ultra-violet region, not characteristic of the substance being analysed but due to impurities remaining after preparation of the spectrophotometric sample. The nature of the irrelevant absorption is usually not known, although the plot against wavelength of the curve for irrelevant absorption can sometimes be determined; it is often found to be a reasonably smooth curve, approximating to a quadratic.

Two approaches are then possible. One is to find a way to remove the substances causing the irrelevant absorption, while still giving a quantitative isolation of the substance being assayed. This can sometimes be done by washing with suitable solvents, by chromatography or by other fractionation procedures. It is unusual to be able to remove the source of interference completely. The second approach is to establish a method of correcting for the irrelevant absorption by a mathematical device. Even with a suitable correction procedure, it is still advisable to keep the irrelevant absorption to the minimum.

One of the most widely used procedures for correcting for irrelevant absorption is that due to Morton and Stubbs,[1] which has been widely described and discussed[2,3,4] and does not require description here. Its fundamental requirement is that a series of wavelengths can be found (three are usually chosen) such that the irrelevant absorption can be joined by a straight line. Shaw[4] has developed an extension of the Morton and Stubbs procedure to correct for irrelevant absorption in solutions of ergosterol extracted from yeasts. His procedure also requires that the irrelevant absorption be linear through the fixation points.

It is obvious that with irrelevant absorption following a quadratic curve, the correction procedure of Morton and Stubbs cannot be applied. For purposes of this paper quadratic curvature is defined as meaning that the relation between absorption and wavelength can be adequately described by a quadratic equation, in the sense that higher terms such as cubic, quartic, etc., if present, are of too small magnitude to affect significantly the estimate of potency. In our experience, this is the most usual type of irrelevant absorption outside the vitamin-A field.

To overcome the limitations of the Morton and Stubbs procedure, we have described a correction procedure for impurities giving quadratic absorption spectra in butyl acetate extracts of griseofulvin.[5] It is based on the development of a series of coefficients highly correlated with the shape of the griseofulvin spectrum in the region of the maximum, but statistically independent of uniform, linear and quadratic absorption spectra.

The advantages of this procedure are—

(*a*) The experimental determination in the irrelevant absorption of artificial "fixation points" that can be joined by a straight line is not required.

(*b*) Provided that the curve for irrelevant absorption can be adequately expressed by an equation not more complex than quadratic, its absolute value and position is not critical.

We have found with griseofulvin solvent extracts of varying origin that the underlying absorption curve is never more complex than a simple quadratic. The precise degree of curvature, the position of the peak and the amount and nature of the irrelevant absorbing material vary considerably from extract to extract. The recommended correction procedure is, however, of general application to these extracts and hence of particular value in routine analysis.

(*c*) Although we have found only quadratic irrelevant-absorption spectra, the method of correlated correction coefficients could be extended, if necessary, to more complex forms of irrelevant absorption, *e.g.*, cubic. The limitation of the method is that forms of irrelevant absorption of complexity equal to or greater than that of the absorption spectrum of the substance being assayed cannot be unequivocally distinguished unless the precise shape of the irrelevant-absorption spectrum is known (see "Discussion" below).

Any number of wavelengths can be used in excess of three, but we have chosen seven. In this way the precision of the corrected estimate when dealing with sharp maxima is considerably improved (see Appendix I).

The calculation of these coefficients and other information about the procedure have been reported previously.[5] This paper describes results obtained by applying the correction to samples of griseofulvin to which have been added artificial sources of irrelevant absorptions.

Experimental

The correction procedure was evaluated by adding ultra-violet absorbing substances to solutions of pure griseofulvin in butyl acetate. The reagents chosen to act as irrelevant absorption had to fulfil the following conditions—

(*a*) they must be sufficiently soluble in butyl acetate to give an absorbance of about 0·7 at 290 mμ in a 1-cm cell,
(*b*) their absorption spectra in butyl acetate solution must be stable over the range 280 to 300 mμ for at least the time required to carry out the experiment,
(*c*) they should not interact with griseofulvin, and
(*d*) they must have absorption spectra of the desired type over the range 280 to 300 mμ.

Of the many substances examined the following were chosen as suitable for our purposes—

(*i*) *p*-nitrophenylacetic acid, which has a nearly linear absorption spectrum (see Table I),
(*ii*) 6-methylquinoline, with an absorption spectrum approximating to a simple curve, in the sense of the definition employed in this paper,
(*iii*) 2-aminoquinoline, which has a markedly curved absorption spectrum with a minimum at 292 to 293 mμ, and
(*iv*) anthrone, which has a more complex absorption spectrum with a small peak at approximately 296 mμ.

Table I

Absorbances of griseofulvin and irrelevant-absorption substances in butyl acetate (mean of two replicates)

Substance	Wavelength						
	288 mμ	290 mμ	292 mμ	294 mμ	296 mμ	298 mμ	300 mμ
Griseofulvin	0·658	0·656	0·559	0·431	0·301	0·209	0·161
p-Nitrophenylacetic acid	0·739	0·660	0·578	0·510	0·441	0·373	0·317
6-Methylquinoline ..	0·639	0·605	0·582	0·545	0·515	0·500	0·464
2-Aminoquinoline ..	0·718	0·641	0·618	0·634	0·680	0·759	0·860
Anthrone..	0·697	0·655	0·634	0·639	0·641	0·618	0·568

p-Nitrophenylacetic acid and 6-methylquinoline have to different degrees the type of quadratic irrelevant-absorption spectra commonly found in quantitative ultra-violet spectrophotometry: 2-aminoquinoline represents a type of curved absorption spectrum that at first sight might appear quadratic, but is shown below to be of more complex nature, whereas anthrone represents a type of curved absorption spectrum quite clearly of more complex nature, having in addition to its general curvature a slight peak in the neighbourhood of the griseofulvin maximum.

PREPARATION OF SOLUTIONS—

In each experiment the material showing irrelevant absorption was added at four levels. For convenience the levels are described as "percentage impurity" and were usually 10, 25, 50 and 75 per cent.

The "percentage-impurity" figures describe the proportions in which the original griseofulvin solution in butyl acetate is mixed with the irrelevant-absorption butyl acetate solution, *e.g.*, 25 per cent. impurity is three parts of griseofulvin solution and one part by volume of irrelevant-absorption solution.

The griseofulvin solution was prepared freshly for each irrelevant-absorption substance by dissolving 9 to 10 mg of purified griseofulvin in 1 litre of butyl acetate. The actual concentration was determined by the seven-point correction procedure by means of coefficients that had been determined previously for the sample of griseofulvin. These solutions of griseofulvin had absorbances between 0·6 and 0·7.

The irrelevant-absorption solutions were prepared by dissolving sufficient of the appropriate substance in butyl acetate to give an absorbance of between 0·6 and 0·7 at 290 mμ.

The various dilutions were prepared in 100-ml grade-A calibrated flasks with grade-A pipettes, by taking the appropriate volume of each solution.

Separate solutions and dilutions were prepared for each experiment, each griseofulvin master solution being used for preparing only one set of irrelevant-absorption dilutions.

TABLE II

RESULTS OBTAINED AT 288 TO 300 mμ

Irrelevant absorption	Percentage of irrelevant absorption	Observed results	Theoretical results
p-Nitrophenylacetic acid	0	9·08, 9·08	9·08
	15	7·41, 7·65	7·71
	25	6·80, 6·80	6·80
	50	4·53, 4·44	4·49
	75	2·21, 2·30	2·25
	100	0·07, 0·15	Nil
6-Methylquinoline	0	9·47, 9·55	9·51
	10	8·70, 8·79	8·56
	25	7·17, 6·96	7·13
	50	4·91, 5·02	4·75
	75	2·82, 2·50	2·38
	100	0·05, 0·12	Nil
2-Aminoquinoline	0	9·11, 9·12	9·11
	10	8·09, 8·13	8·20
	25	6·36, 6·79	6·84
	50	3·79, 3·75	4·56
	75	0·83, 0·85	2·28
	100	− 1·65, − 1·74	Nil
Anthrone	0	9·27, 9·31	9·29
	10	7·98, 8·08	8·36
	25	6·10, 6·15	6·97
	50	7·37, 7·45	4·65
	75	5·50, 5·33	2·32
	100	2·58, 2·61	Nil

NOTE—Figures represent μg of griseofulvin per ml in solution.

DETERMINATION OF ABSORBANCES—

The spectrophotometric measurements were made on a Unicam SP500 quartz spectrophotometer. Each dilution was measured at the seven wavelengths in two separate 1-cm silica cells against butyl acetate, thus giving a measure of the precision of the method.

Measurements were made at seven wavelengths, 288 to 300 mμ, at intervals of 2 mμ.

Results

The results obtained are shown in Table II.

From Table II it will be seen that the experimental results with *p*-nitrophenylacetic acid and 6-methylquinoline are in good agreement with the theoretical results irrespective of the level of irrelevant absorption.

With 2-aminoquinoline the results obtained are markedly discrepant at the 50 per cent. impurity level. For anthrone the discrepancy increases with increasing levels of impurity. With both the existence of an appreciable level of "apparent griseofulvin," negative or positive, in the solution containing no griseofulvin indicates that both substances have more complex absorption spectra than can be approximated by a quadratic. With *p*-nitrophenylacetic acid and 6-methylquinoline (Table II) the solutions containing no griseofulvin give (within experimental error) the correct answer, namely zero, and this establishes that the coefficients employed are in fact orthogonal to their absorption spectra. In other words, the absorption spectra of these substances can be adequately represented by a quadratic, as required for this particular correction procedure.

These results show that impurities with quadratic irrelevant-absorption spectra can be accurately accounted for by the correction procedure, even when the amount of irrelevant absorption is two or three times that due to the substance being assayed.

Discussion

The correction procedure as described[5] is capable of dealing with impurities having quadratic absorption spectra. However, this type of correction can be extended to more complex cases in two ways. These are as follows—

(1) When the irrelevant-absorption spectrum is more complex than a quadratic, though still of lower order than that of the substance being assayed, the principles of the correction remain the same and involve constructing a set of coefficients highly correlated with the absorption spectrum of the substance being assayed, but statistically independent over the wavelengths employed of linear, quadratic, cubic and so on, spectra as far as is required. Full details of the manner of establishing the order of complexity required to define the irrelevant-absorption spectrum are given in Appendix II.

(2) When the irrelevant absorption is known to be due to a substance, or more than one, whose absorption spectra are accurately known, in which event, coefficients are calculated that over the wavelengths employed are statistically independent of the irrelevant-absorption spectrum, but are most highly correlated with the absorption spectrum of the substance being assayed. An example of this approach applied to the 2-aminoquinoline and anthrone mixtures with griseofulvin (previously shown in Table II) is given in Table III.

Table III

Results from Table II recalculated by method two outlined in the discussion

Irrelevant absorption	Percentage of irrelevant absorption	Recalculated results	Theoretical results
2-Aminoquinoline	0	9·12, 9·11	9·11
	10	8·21, 8·21	8·20
	25	6·87, 6·88	6·84
	50	4·53, 4·52	4·56
	75	2·26, 2·28	2·28
	100	0·57, − 0·57	Nil
Anthrone	0	9·30, 9·28	9·29
	10	8·35, 8·38	8·36
	25	6·96, 6·99	6·97
	50	5·27, 5·30	4·65
	75	2·15, 2·20	2·32
	100	0·13, − 0·13	Nil

With 2-aminoquinoline the results of this alternative correction procedure are highly satisfactory and show that a simple additive relationshp holds between the absorbances of the constituents.

With anthrone, however, agreement, though improved, is not complete. Although we have not studied this phenomenon further, the results suggest that the absorbances are not strictly additive, some interaction occurring.

By extension of this principle, the simultaneous assay of non-interacting, binary, tertiary, etc., mixtures of substances with characteristic ultra-violet absorption is possible even when the peaks of the substances concerned are not sufficiently separated to permit their separate assay by their respective peaks.

We acknowledge with thanks the technical assistance of Miss I. Thompson.

APPENDIX I

LOSS OF PRECISION WITH INCREASING COMPLEXITY OF CORRECTIONS—

The absorbance of the sample at any given wavelength, λ, is assumed to be the sum of the absorbance of the griseofulvin in the sample and irrelevant absorbance of the impurity, the latter being given by an n^{th} degree polynomial function in λ, whose constants are to be determined from the internal evidence of the assay.

At the given wavelength λ let—

S = absorbance of pure standard griseofulvin solution,
U = absorbance of sample,
p = ratio of griseofulvin in sample compared with the standard,
x_m = the value of a polynomial of m^{th} degree in λ, $m \leqslant n$, and
a_m = constant defining the m^{th} polynomial of irrelevant absorption.

Then—

$$U = p\,S + a_0 x_0 + a_1 x_1 + a_2 x_2 \ldots + a_m x_m.$$

For convenience the x polynomials are orthogonal and are defined as follows, summation being over all wavelengths employed in the assay—

$$\Sigma\, x_{m_1} x_{m_2} = 0,\ m_1 \neq m_2$$
$$= 1,\ m_1 = m_2$$

The normal equations, in matrix form, are then as follows—

$$\begin{bmatrix} \Sigma S^2 & \Sigma S x_0 & \Sigma S x_1 & \Sigma S x_2 \ldots & \Sigma S x_n \\ \Sigma S x_0 & 1 & 0 & 0 \ldots & 0 \\ \Sigma S x_1 & 0 & 1 & 0 \ldots & 0 \\ \Sigma S x_2 & 0 & 0 & 1 \ldots & 0 \\ \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot \\ \Sigma S x_n & 0 & 0 & 0 \ldots & 1 \end{bmatrix} \begin{bmatrix} p \\ a_0 \\ a_1 \\ a_2 \\ \cdot \\ \cdot \\ \cdot \\ a_n \end{bmatrix} = \begin{bmatrix} \Sigma SU \\ \Sigma U x_0 \\ \Sigma U x_1 \\ \Sigma U x_2 \\ \cdot \\ \cdot \\ \cdot \\ \Sigma U x_n \end{bmatrix}$$

The solution of the normal equations by inversion of the matrix is thus—

$$\frac{1}{D}\begin{bmatrix} 1 & -\Sigma S x_0 & -\Sigma S x_1 & -\Sigma S x_2 \ldots & -\Sigma S x_n \\ -\Sigma S x_0 & D+(\Sigma S x_0)^2 & \Sigma S x_0 \Sigma S x_1 & \Sigma S x_0 \Sigma S x_2 \ldots & \Sigma S x_0 \Sigma S x_n \\ -\Sigma S x_1 & \Sigma S x_0 \Sigma S x_1 & D+(\Sigma S x_1)^2 & \Sigma S x_1 \Sigma S x_2 \ldots & \Sigma S x_1 \Sigma S x_n \\ -\Sigma S x_2 & \Sigma S x_0 \Sigma S x_2 & \Sigma S x_1 \Sigma S x_2 & D+(\Sigma S x_2)^2 \ldots & \Sigma S x_2 \Sigma S x_n \\ \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot \\ -\Sigma S x_n & \Sigma S x_0 \Sigma S x_n & \Sigma S x_1 \Sigma S x_n & \Sigma S x_2 \Sigma S x_n \ldots & D+(\Sigma S x_n)^2 \end{bmatrix} \begin{bmatrix} \Sigma SU \\ \Sigma U x_0 \\ \Sigma U x_1 \\ \Sigma U x_2 \\ \cdot \\ \cdot \\ \cdot \\ \Sigma U x_n \end{bmatrix} = \begin{bmatrix} p \\ a_0 \\ a_1 \\ a_2 \\ \cdot \\ \cdot \\ \cdot \\ a_n \end{bmatrix}$$

where $D = \Sigma S^2 - (\Sigma S x_0)^2 - (\Sigma S x_1)^2 - (\Sigma S x_2)^2 \ldots - (\Sigma S x_n)^2$.

Now the variance of p, the estimate of griseofulvin concentration in the sample, is proportional to $1/D$.

From this relationship the following facts can be deduced—

(1) In general a loss of precision will occur for each additional term of the polynomial required to describe the irrelevant-absorption spectrum.

(2) Hence the number of terms employed should not be excessive and should not be taken to the point where gain in accuracy is more than offset by loss in precision.

(3) If the complexity of the irrelevant-absorption spectrum is of the same order as that of the griseofulvin curve, say of m^{th} degree, so that the griseofulvin curve could be defined by an m^{th} degree polynomial, then D will be zero and the method fails. Clearly the method will also fail even when the irrelevant-absorption spectrum is not particularly complex, say of order m, if less wavelength settings than $m + 2$ are employed.

(4) In view of the loss of precision with additional correction terms, an increased number of absorbance readings is required to reduce the error of the method. This may be done either by replicatory readings at the minimum number of wavelength settings dictated by the complexity of the correction or by reading at more wavelength settings.

APPENDIX II

DETERMINATION OF THE DEGREE OF THE IRRELEVANT ABSORPTION—

The orthogonal polynomials x_0, x_1, x_2, etc., employed in Appendix I, though orthogonal to each other, are not orthogonal to the griseofulvin curve. To ascertain which of these components is introducing significant bias into the results and to determine the magnitude of the bias introduced, there is a considerable advantage in using functions mutually orthogonal and orthogonal to the griseofulvin-absorption spectrum over the wavelengths employed. These functions are designed to estimate successively the uncorrect griseofulvin estimate, correction for uniform absorption, additional correction for linear absorption and so on.

With the same nomenclature as in Appendix I, the following functions have the desired properties—

$$S$$

$$S - x_0 \frac{\Sigma S^2}{\Sigma S x_0}$$

$$S - x_0 \Sigma S x_0 - x_1 \left\{ \frac{\Sigma S^2 - (\Sigma S x_0)^2}{\Sigma S x_1} \right\}$$

$$S - x_0 \Sigma S x_0 - x_1 \Sigma S x_1 - x_2 \left\{ \frac{\Sigma S^2 - (\Sigma S x_0)^2 - (\Sigma S x_1)^2}{\Sigma S x_2} \right\}$$

$$S - x_0 \Sigma S x_0 - x_1 \Sigma S x_1 - x_2 \Sigma S x_2 - x_3 \left\{ \frac{\Sigma S^2 - (\Sigma S x_0)^2 - (\Sigma S x_1)^2 - (\Sigma S x_2)^2}{\Sigma S x_3} \right\}$$

and so on.

In practice the coefficients corresponding to these functions would not be evaluated. The various sums involved ΣS^2, $\Sigma S x_0$, $\Sigma S x_1$, $\Sigma S x_2$, etc., and the corresponding sums ΣSU, $\Sigma U x_1$, $\Sigma U x_2$, $\Sigma U x_3$, etc., would be evaluated from the known values of S and U and from suitable whole number multiples of x_0, x_1, x_2, etc.,[6] and these sums entered into the following formulae derived from the aforementioned functions.

Let p = uncorrected griseofulvin estimate,
let Δp_0 = correction to be added for irrelevant uniform absorption,
let Δp_1 = correction to be added for irrelevant linear absorption,
let Δp_2 = correction to be added for irrelevant quadratic absorption,
let Δp_3 = correction to be added for irrelevant cubic absorption,
etc.

Then—

$$p = \frac{\Sigma SU}{\Sigma S^2}$$

$$\Delta p_0 = \frac{(\Sigma Sx_0)^2 \left\{ \Sigma SU - \frac{\Sigma Ux_0 \, \Sigma S^2}{\Sigma Sx_0} \right\}}{\Sigma S^2 \{\Sigma S^2 - (\Sigma Sx_0)^2\}}$$

$$\Delta p_1 = \frac{(\Sigma Sx_1)^2 \left[\Sigma SU - \Sigma Ux_0 \, \Sigma Sx_0 - \frac{\Sigma Ux_1}{\Sigma Sx_1} \left\{ \Sigma S^2 - (\Sigma Sx_0)^2 \right\} \right]}{\{\Sigma S^2 - (\Sigma Sx_0)^2\}\{\Sigma S^2 - (\Sigma Sx_0)^2 - (\Sigma Sx_1)^2\}}$$

$$\Delta p_2 = \frac{(\Sigma Sx_2)^2 \left[\Sigma SU - \Sigma Ux_0 \, \Sigma Sx_0 - \Sigma Ux_1 \, \Sigma Sx_1 - \frac{\Sigma Ux_2}{\Sigma Sx_2} \left\{ \Sigma S^2 - (\Sigma Sx_0)^2 - (\Sigma Sx_1)^2 \right\} \right]}{\{\Sigma S^2 - (\Sigma Sx_0)^2 - (\Sigma Sx_1)^2\} \{\Sigma S^2 - (\Sigma Sx_0)^2 - (\Sigma Sx_1)^2 - (\Sigma Sx_2)^2\}}$$

etc.

And the sum of squares attributable to each correction may be expressed as follows—

for p : $p\Sigma SU$

for Δp_0: $\Delta p_0 \left[\Sigma SU - \frac{\Sigma Ux_0 \, \Sigma S^2}{\Sigma Sx_0} \right]$

for Δp_1: $\Delta p_1 \left[\Sigma SU - \Sigma Ux_0 \, \Sigma Sx_0 - \frac{\Sigma Ux_1}{\Sigma Sx_1} \left\{ \Sigma S^2 - (\Sigma Sx_0)^2 \right\} \right]$

for Δp_2: $\Delta p_2 \left[\Sigma SU - \Sigma Ux_0 \, \Sigma Sx_0 - \Sigma Ux_1 \, \Sigma Sx_1 - \frac{\Sigma Ux_2}{\Sigma Sx_2} \left\{ \Sigma S^2 - (\Sigma Sx_0)^2 - (\Sigma Sx_1)^2 \right\} \right]$

etc.

In this manner an analysis of variance, centred at zero and not at the mean as usual, may be performed, in which the total variation in the absorbances recorded for the sample may be subdivided into categories attributable to the uncorrected potency estimate, correction for uniform absorption, correction for linear absorption, correction for quadratic absorption and so on.

The variance attributable to each source may be compared with the random error in the readings, and only those corrections that are significant need be considered as introducing significant bias into the result. Such an analysis is summarised in our previous paper.[5]

This process carried out on a suitably representative selection of the samples concerned will indicate the order of correction required. Coefficients suitable for routine use may then be introduced.[5]

References

1. Morton, R. A., and Stubbs, A. L., *Analyst*, 1946, **71**, 348.
2. Gridgeman, N. T., *Ibid.*, 1951, **76**, 449.
3. Bagnall, H. H., and Stock, F. G., *J. Pharm. Pharmacol.*, 1952, **4**, 81.
4. Shaw, W. H. C., and Jefferies, J. P., *Analyst*, 1953, **78**, 519.
5. Ashton, G. C., and Tootill, J. P. R., *Ibid.*, 1956, **81**, 225.
6. Fisher, R. A., and Yates, F., "Statistical Tables for Biological, Agricultural and Medical Research," Fourth Edition, Oliver & Boyd, Edinburgh, 1953, p. 80 *et seq.*

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A Modified Ethylenediaminetetra-acetic Acid - Tannic Acid Procedure for the Determination of Niobium, Tantalum and Titanium Together in Minerals

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Niobium, tantalum and titanium are quantitatively precipitated together and separated from most of the associated elements by tannic acid from oxalate solutions in the presence of ethylenediaminetetra-acetic acid at a pH of about 4·5. Tin and antimony interfere in the procedure by their quantitative precipitation. Methods of separation from these elements are indicated. Tungsten is partly thrown down in the presence of niobium, tantalum and titanium, but it has little effect when present in amounts less than 2 per cent. Single precipitation gives a good separation, so that the method is straightforward and simple, while being applicable to a variety of minerals.

Atkinson, Steigman and Hiskey,[1] reviewing the main separative reactions involved in the analyses of niobium, tantalum and titanium minerals by the Schoeller method, have pointed out the striking lack of agreement even in the values for the total oxides obtained by different workers. The chief reason for this disagreement is the number of operations involved and the necessity for repetitive working with precipitates obtained at different stages.

The works of Schwarzenbach and Přibil show that most of the metals associated with these elements in their minerals form complexes with ethylenediaminetetra-acetic acid (EDTA). Titanium[2,3] is reported to form only a very weak complex, whilst there is no mention of the complexes of niobium and tantalum. This is to be expected, since these two elements do not exist as stable cations in solutions. Hiskey and Batik[4] have used EDTA in combination with chelating agents "chel 153" and "chel 242" to separate iron and manganese from niobium and tantalum, which are precipitated hydrolytically by guanidine carbonate at pH 12.

The present work describes the possibility of precipitating these three elements with tannic acid in the presence of EDTA, which masks the associated elements.

Experimental

Materials—

A Cambridge bench-type pH meter was used for all pH measurements.

Niobium, tantalum and titanium solutions were prepared from weighed amounts of ignited Specpure oxides that were separately fused with potassium bisulphate, the melt being dissolved in oxalic and hydrochloric acids and made up to definite volumes.

A stock solution of niobium, tantalum and titanium was prepared from a sample of columbite - tantalite that had been analysed for total earth acids and titania ($Nb_2O_5 + Ta_2O_5$ = 78·1 per cent.; TiO_2 = 1·4 per cent.) and was free from tungsten; the ore was subjected to bisulphate fusion and tartaric acid hydrolysis twice. The final hydrolysis product was dissolved in a mixture of oxalic and hydrochloric acids and made up to a definite volume. A 10-ml aliquot of the solution was found to contain 44·0 mg of mixed oxides (by the Schoeller method).

A 10 per cent. w/v aqueous solution of disodium ethylenediaminetetra-acetate (EDTA) was used. All other reagents were either Merck G. R. or AnalaR.

Effect of EDTA on the precipitation of niobium, tantalum and titanium with tannic acid—

Aliquots of the solutions of the Specpure oxides were separately precipitated with tannic acid in the usual way in the presence of 20 ml of EDTA solution. The tannic acid precipitates were filtered off, washed, ignited to oxides and weighed. The results given in Table I show that EDTA does not interfere in their precipitation.

TABLE I

PRECIPITATION OF NIOBIUM, TANTALUM AND TITANIUM WITH TANNIC ACID IN THE PRESENCE OF EDTA

Element	Oxides taken, mg	Oxides found, mg	Difference, mg
Niobium	33·0	32·9	− 0·1
	66·0	66·1	+ 0·1
Tantalum	33·5	33·6	+ 0·1
	67·0	66·8	− 0·2
Titanium	35·0	35·2	+ 0·2
	70·0	70·2	+ 0·2

EFFECT OF pH—

Schoeller and Powell[5] have recommended the addition of ammonia solution to a solution of the mixed oxides in oxalate or tartrate containing tannic acid until a part of the iron is also precipitated (indicated by its mauve colour) and then redissolving the iron preferentially by the addition of a few drops of mineral acid. They have not stipulated any particular pH for the precipitation. Bhattacharya[6] has recommended an initial pH of 4·0 for tantalum and 4·4 for niobium when they are precipitated from 2 per cent. ammonium oxalate solution by a fresh solution of tannic acid. Titanium is precipitated from a solution of pH 4·2.

The procedure described below was used to find the lowest pH at which these elements were precipitated together.

To an aliquot of the stock solution in a 600-ml beaker were added 60 to 70 ml of saturated ammonium chloride solution, 0·5 g of tartaric acid (to prevent the formation of any hydrated oxide during the adjustment of pH) and 25 ml of EDTA solution. This was diluted to 400 ml, the pH was adjusted to the required value and the solution was brought to the boil. To the boiling solution were added 10 ml of 10 per cent. tannic acid solution, also adjusted to the same pH as that of the solution, and boiling was continued for a couple of minutes. The precipitate was allowed to settle for half an hour, the solution being kept hot, collected on a Whatman No. 540 filter-paper, washed with a wash liquid containing 5 g of tannic acid and 100 ml of saturated ammonium chloride solution per litre and adjusted to the same pH as that of the solution, ignited and weighed. The results in Table II show that precipitation is quantitative at pH 4·0 and above.

TABLE II

EFFECT OF pH

Amount of "mixed oxides" taken = 44·0 mg

pH	Oxides found, mg	Difference, mg
4·6	44·1	+ 0·1
4·4	44·0	Nil
4·2	43·8	− 0·2
4·0	43·9	− 0·1
3·8	40·2	− 3·8
3·6	30·8	− 13·2

INFLUENCE OF ASSOCIATED ELEMENTS—

Elements usually associated with niobium, tantalum and titanium in their ores were separately added to different aliquots of the stock solution and the "mixed oxides" were precipitated at pH 4·5. The precipitates, after being allowed to settle, were filtered off, washed back into the original beakers and churned well with the wash liquid, which in addition to tannic acid and ammonium chloride contained 1 per cent. of EDTA solution, and re-filtered on the original filter-papers. They were washed twice with the same wash liquid and finally with wash liquid not containing EDTA. The precipitates were ignited and weighed as oxides. The results are shown in Table III.

These results show that most of the associated elements are effectively masked by EDTA. Only tin and antimony are quantitatively precipitated. Tungsten is partly thrown down in the presence of the earth acids.

Before the method was applied to samples, the range of pH within which niobium, tantalum and titanium could be separated quantitatively from associated elements was investigated for synthetic mixtures. Tin, antimony and tungsten were not taken in the impurities. In these mixtures the proportion of the "mixed oxides" to the other elements was varied between 22 and 88 per cent. of the sample. The separations were good between pH 4 and 5. Even at pH 5·4, only when the impurities were high wese the mixed oxides obtained slightly contaminated. In the absence of EDTA, even at pH 4·5 impurities were co-precipitated to the extent of 4 to 5 per cent.

TABLE III

INFLUENCE OF ASSOCIATED ELEMENTS

Amount of "mixed oxides" taken = 44·0 mg

Impurity added	Amount of impurity as oxides, mg	Total oxides obtained, mg	Difference, mg
Calcium chloride	20·0	44·0	nil
Magnesium nitrate	23·0	44·1	+ 0·1
Ferric nitrate	50·0 (Fe_2O_3)	44·1	+ 0·1
Ferrous ammonium sulphate	53·2 (FeO)	44·0	nil
Manganous chloride	48·0	44·1	+ 0·1
Rare-earths chloride	25·6	43·9	− 0·1
Thorium nitrate	20·0	44·0	nil
Uranyl nitrate	32·0	44·1	+ 0·1
Zirconyl chloride	12·3	44·0	nil
Aluminium nitrate	20·3	44·2	+ 0·2
Bismuth nitrate	52·0	44·1	+ 0·1
Lead nitrate	10·0	43·9	− 0·1
Stannous chloride	5·2	49·1	+ 5·1
Stannous chloride	10·4	54·3	+ 10·3
Antimonyl chloride	43·0	86·9	+ 42·9
Antimonyl chloride	21·5	65·4	+ 21·4
Sodium tungstate	15·0	49·8	+ 5·8
Sodium tungstate	4·3	44·8	+ 0·8
Sodium tungstate	2·1	44·1	+ 0·1

It was also found that the final pH of the solution adjusted itself to about 4·5 when a freshly prepared solution of tannic acid (50 ml of 10 per cent. solution for a maximum amount of 0·25 g of the oxides) was added to the boiling oxalate solution of the mixed oxides and impurities, which was initially adjusted to a pH of about 5·5. This procedure had the additional advantage that the precipitate was compact.

The method was finally verified for synthetic and previously analysed samples. The synthetic samples were prepared by mixing known amounts of the "mixed oxides" with different amounts of an oxide mixture of the impurities to give a final sample of 0·25 g. The composition of the oxide mixture of the impurities was: MnO, 20%; Fe_2O_3, 25%; Bi_2O_3, 5%; PbO, 2%; rare-earths oxides, 15%; U_3O_8, 20%; ThO_2, 5%; ZrO_2, 3%; Al_2O_3, 2%; CaO, 2%; and MgO, 1%.

PROCEDURE—

Fuse 0·25 g of the sample with 8 to 10 g of potassium bisulphate thoroughly to crystallisation. Dissolve the cooled melt by warming in a mixture of about 30 ml of saturated oxalic acid solution, 10 ml of concentrated hydrochloric acid and 100 ml of water. Filter through Whatman No. 42 filter-paper and wash the residue thoroughly with water containing oxalic acid and a few drops of hydrochloric acid. To the filtrate add 60 to 70 ml of saturated ammonium chloride solution, 0·5 g of tartaric acid and 25 ml of EDTA solution and dilute to 400 ml. Adjust the pH to between 5 and 6 (this may be done with a little experience, bromocresol green or purple being used), and heat to boiling. To the boiling solution add 50 ml of 10 per cent. tannic acid solution and complete the determination as described under the study of the effect of associated elements.

RESULTS

The results obtained for a number of samples are given in Table IV.

TABLE IV

ANALYSIS OF SYNTHETIC SAMPLES AND MINERALS

Sample No.	Nature	Mixed oxides taken, %	Mixed oxides found, %	Difference, %
1	Synthetic samples	80·9	81·0	+ 0·1
2		40·0	39·6	− 0·4
3		23·0	23·3	+ 0·3
4		6·0	6·3	+ 0·3
5	Ilmenite	39·0	38·7	− 0·3
6	Samarskite	44·0	44·4	+ 0·4
7	Rutile	96·8	96·9	+ 0·1
8	Columbite - tantalite	76·0	76·4	+ 0·4

NOTE—Samples No. 5 to 8 were previously analysed by the Schoeller method.

DISCUSSION

Among the elements usually associated with niobium, tantalum and titanium in their minerals, only tin, antimony and tungsten interfere in the present modified procedure. Of these, tin and antimony, when present in solution, interfere by their quantitative precipitation with tannic acid and hence they have to be removed by the usual hydrogen sulphide separation. Antimony is reported only in some rare samples of stibiotantalites. When present, this element, unlike tin, can be initially separated by means of hydrogen sulphide from oxalate solutions.

Tin is the element that offers serious limitations to the general applicability of the method. Tin, in a sample, can be tested for by the sensitive Meissner test.[7,8] If it is absent, the method becomes directly applicable. If present, it may be collected together with the "mixed oxides" and the figure for total oxides corrected after separating tin by hydrogen sulphide from the fused oxides dissolved in tartaric acid.

Tungsten alone is not precipitated by tannic acid, but in the presence of earth acids is partly precipitated.[9] In our experiments with 4 per cent. of tungstic oxide present, only a fraction of a milligram could be recovered. Usually the tungsten content of niobium-tantalum minerals is small, the maximum being 2·25 per cent.[10] In these concentrations it does not affect the final results appreciably, even though spectroscopic analysis of the final oxides obtained in our experiments revealed its presence in traces.

On the whole, the method is simple and direct, while being applicable to a variety of minerals of niobium, tantalum and titanium.

The authors thank Dr. Jagdish Shankar for his keen interest in the work.

REFERENCES

1. Atkinson, R. H., Steigman, J., and Hiskey, C. F., *Anal. Chem.*, 1952, **24**, 477.
2. Pickering, W. F., *Anal. Chim. Acta*, 1953, **9**, 324.
3. Přibil, R., *Coll. Czech. Chem. Comm.*, 1952, **18**, 305 (abstracted in *Chem. Listy*, 1952, **46**, 218).
4. Hiskey, C. F., and Batik, A. L., *Anal. Chem.*, 1953, **25**, 823.
5. Schoeller, W. R., and Powell, A. R., "The Analysis of Minerals and Ores of the Rarer Elements," Charles Griffin & Co. Ltd., London, 1940, p. 98.
6. Bhattacharya, H., *J. Indian Chem. Soc.*, 1952, **29**, 871; **29**, 891.
7. Meissner, H., *Z. anal. Chem.*, 1930, **80**, 247.
8. Treadwell, F. P., and Hall, W. T., "Analytical Chemistry," John Wiley & Sons Inc., New York, 1947, Volume I, p. 178.
9. Schoeller, W. R., and Jahn, C., *Analyst*, 1934, **59**, 465.
10. Palache, C., Berman, H., and Frondel, C., "Dana's System of Mineralogy," John Wiley & Sons Inc., New York, 1946, Volume I, pp. 745 to 808.

CHEMISTRY DIVISION
ATOMIC ENERGY ESTABLISHMENT
BOMBAY 28

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Notes

A TENTATIVE METHOD FOR THE DETERMINATION OF THE GRADE STRENGTH OF AGARS

A NEED has long been felt for a standard method of grading agars in regard to their jellying powers. In order to avoid the premature proposal of a method that might have been found unsatisfactory in practice, this matter was deferred until sufficient research had been conducted on the jellying properties of agar. It is now considered that the work on agar being carried out in the B.F.M.I.R.A. laboratories has reached a stage where a satisfactory tentative method can be drawn up.

The proposed grading method is based on a determination of the percentage of agar necessary to produce an agar - water jelly of a given strength when prepared under standard conditions. The standard jelly strength chosen is one of 75 g, for a deflection of 20° on the F.I.R.A. jelly tester* (the improved version of the older B.A.R. instrument). Jelly tests are made at two concentrations of agar (0·5 and 1·0 per cent.) and the concentration required to produce a jelly with a strength of 75 g is found by interpolation. The grade strength is conveniently expressed as the number of grams of jelly of the standard strength obtainable from 1 g of agar. This is given by dividing the percentage of agar in the standard-strength jelly into 100.

EXPERIMENTAL

PREPARATION OF THE JELLIES—

Weigh out 2·5-g and 5·0-g portions of a representative sample of the agar and place each in 500 ml of water contained in a tared 1-litre beaker with a glass stirring rod. With shredded agar allow the agar to soak in the water for at least 2 hours (it is often convenient to soak overnight) before boiling; no soaking time is required for powdered agars.

Heat the mixture to boiling, stirring when necessary to prevent powdered agars from sticking to the bottom or sides of the beaker, and simmer gently for 5 minutes.

At the end of the boiling period, add hot water, with thorough stirring, until the net weight of solution is 500 g ($\pm$ 0·5 g) and pour the solution into three 2-inch square jelly boxes (as supplied for use with the F.I.R.A. jelly tester), filling the boxes completely. The pouring temperature is not critical, but should not be allowed to fall appreciably below 50° C to ensure that premature setting does not occur. Cover the surface of the solution in the jelly boxes with 2-inch squares of moisture-proof cellulose film or waxed paper.

Allow the jellies to set in a bath of running water and then store them overnight in a refrigerator at 5° C. Leaving the jellies in the refrigerator for 3 nights does not affect the jelly strength.

TESTING—

Remove the cellulose film or waxed paper and test the jellies on the F.I.R.A. jelly tester at a temperature between 5° and 8° C (checked by inserting a thermometer into the jelly immediately after completing the test). Run water into the bucket of the jelly tester at a standard rate of 100 ml per minute and measure the quantity required to produce a spade deflection of 20°. Carry out a blank test with water in the jelly box and subtract the result from the readings for the jellies to give the jelly strength in grams. Calculate the mean value for each batch of three jellies and determine the concentration of agar required to give a jelly strength of 75 g by interpolation. Divide the concentration found (expressed as a percentage) into 100 to give the grade strength of the agar.

NOTES—

1. It is normally desirable to grade raw materials under conditions as close as possible to those under which the material is to be used. Agar has so many uses that it is considered best to choose the simplest conditions for the standard test, and so a simple agar - water jelly has been employed. Research carried out in the B.F.M.I.R.A. laboratories on the jellying properties of agar suggests that the relative strengths of two samples of agar remain reasonably constant under different conditions of acidity and sugar concentration (although this is not always correct for other than true agars, *e.g.*, Danish agar).

* The F.I.R.A. jelly tester is manufactured and supplied by Messrs. H. A. Gaydon & Co. Ltd., 93 Lansdowne Road, Croydon, Surrey.

2. The standard jelly strength (75 g) and the concentrations of agar (0·5 and 1·0 per cent.) chosen for this test are designed to utilise a range over which the jelly strength - concentration relationship is reasonably linear for practically all samples of agar. Only with exceptional samples is a jelly containing less than 0·5 or more than 1·0 per cent. of agar likely to have a jelly strength of 75 g. In such cases it might be necessary to make another test at a different concentration of agar.

3. A deflection of 20° on the F.I.R.A. jelly tester is used, instead of the 30° recommended for pectin and gelatin, as it has been found that agar jellies often split during testing before a deflection of 30° has been reached, and also because measurements of the jelly strength are less sensitive to temperature variations if taken at the smaller deflection.

4. The jelly strength varies slightly with the temperature of the jelly at the time of testing, but, provided the suggested temperature limits are observed, the errors are negligible. No difficulty is experienced in practice if not more than six jellies are removed from the refrigerator at one time.

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British Food Manufacturing Industries Research Association
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THE USE OF ALKALI HYDROXIDES FOR THE SEPARATION OF THE COPPER AND ARSENIC GROUPS IN QUALITATIVE INORGANIC ANALYSIS

Holness and Trewick[1] (compare Holness[2]) have claimed that "a 1 per cent. aqueous solution of lithium hydroxide containing in it 5 per cent. of potassium nitrate" possesses a number of advantages over the other alkali hydroxides in the separation of the copper and arsenic groups in qualitative analysis; in particular, they state[1,2] that stannous sulphide is dissolved at the boiling point. Nutten[3] regards the lithium hydroxide separation as "one of the outstanding contributions to qualitative analysis in recent years"; he states that his "own experience confirms this opinion" and that "it has solved a long-standing problem in qualitative analysis." James and Woodward,[4] as a result of a comprehensive study, conclude "that lithium hydroxide possesses no peculiar merits in comparison with sodium and potassium hydroxides; that if mercury and group 2B metals are present together, mercury must be sought in both group 2A and 2B; that tin^{II} should be oxidised before precipitation; and that potassium hydroxide of concentration about 0·5 *N* is most suitable for the separation."

In view of this serious difference of opinion about the merits of lithium hydroxide, we wish to draw attention to our own experiments, which were completed before those of James and Woodward, and to state that the applications of our results to qualitative analysis were published in 1954.[5] In general, the results of our systematic investigation were similar to those of James and Woodward, although we prefer to employ 2 *N* potassium hydroxide (compare Curtman,[6,7] who uses 6 *M* potassium hydroxide). Although the solubilities of sulphides depend upon numerous factors (conditions of precipitation, particle size, etc.), we should like to record that stannous sulphide, precipitated under the usual conditions of qualitative analysis, has the following solubilities in 100 ml of boiling alkali: 0·5 *N* potassium hydroxide, 0·21 g; *N* potassium hydroxide, 0·26 g; 2 *N* potassium hydroxide, 0·31 g. We would draw attention to the fact that a solution of stannous sulphide in caustic alkali (equivalent to a solution of a thiostannite and a stannite) is a powerful reducing agent and will reduce *inter alia* thioarsenites and thioantimonites to elementary arsenic and antimony, respectively (compare Curtman and Marcus[8]). For this and other reasons it is essential to oxidise tin^{II} with hydrogen peroxide (or with bromine water) before precipitation with hydrogen sulphide; in the presence of arsenic^{III} and antimony^{III} preferential oxidation of tin^{II} occurs and, provided the amount of hydrogen peroxide is not excessive, relatively little oxidation of arsenic^{III} and antimony^{III} occurs. We thus differ from Curtman and Marcus,[8] who reject the use of hydrogen peroxide for the oxidation of stannous tin and prefer to use sulphur dioxide for this purpose; sulphur dioxide possesses many obvious disadvantages. We conclude that lithium hydroxide solution possesses no superiority over potassium hydroxide solution in the separation of the copper and arsenic groups: apart from the question of cost, the use of the lithium hydroxide reagent should be discouraged, since it must be protected from atmospheric carbon dioxide to prevent the precipitation of lithium carbonate.

References

1. Holness, H., and Trewick, R. F. G., *Analyst*, 1950, **75**, 276.
2. Holness, H., "Inorganic Qualitative Analysis. Semi-micro Methods," Sir Isaac Pitman and Sons Ltd., London, 1954, p. 51.
3. Nutten, A. J., *The Industrial Chemist*, 1955, **31**, 367.
4. James, C. F., and Woodward, P., *Analyst*, 1955, **80**, 825.
5. Vogel, A. I., "A Text-Book of Macro and Semimicro Qualitative Inorganic Analysis," Fourth Edition, Longmans, Green and Co. Ltd., London, 1954, pp. 515, 521, 522 *et seq.*
6. Curtman, L. J., "Qualitative Chemical Analysis," The Macmillan Company Inc., New York, 1938, pp. 269, 274.
7. ——, "Introduction to Semimicro Qualitative Chemical Analysis," The Macmillan Company Inc., New York, 1950, p. 262.
8. Curtman, L. J., and Marcus, J. K., *J. Amer. Chem. Soc.*, 1914, **36**, 1101.

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VOLUMETRIC METHOD FOR SIMULTANEOUS DETERMINATION OF CADMIUM AND CHLORIDE

For investigations of the activity of cadmium chloride in molten binary mixtures of cadmium chloride and alkali-metal chlorides carried out in this laboratory,[1,2] methods were required for determining the mole fraction of each component. A standard procedure is the gravimetric determination of the chloride and a separate electrolytic determination of cadmium. When numerous analyses are required with an accuracy of 0·1 per cent., the standard method becomes cumbersome, as chloride must be removed from the sample by heating to fumes with concentrated sulphuric acid before electrolysis. Methods for depositing cadmium in the presence of chloride have been suggested,[3] but are generally less reliable than that in which the chloride has been removed. The method depending upon consecutive volumetric analyses for chloride and cadmium on the same sample is less time-consuming than the standard method and permits the evaluation of molar composition of the mixed chlorides without knowing the weight of the sample. Coetzee[4] has shown that under the correct conditions, cadmium hydroxide can be precipitated quantitatively. The threshold value of pH for precipitation of cadmium as hydroxide[5] is 7·6 and the precipitation may be considered complete at pH 10. Thus, thymolphthalein, with the pH range 9·3 to 10·5, is a suitable end-point indicator for the precipitation of cadmium as hydroxide. The formation of basic cadmium compounds can be prevented by carrying out the titration above 70° C, and since cadmium tends to form complexes with chloride ions in solution, chloride must be absent when sodium hydroxide is added.

Method

Dissolve a sample of cadmium chloride - alkali-metal chloride mixture in water to form a 0·5 to 1 per cent. w/v solution. Make a 25-ml aliquot neutral to methyl red by adding 0·05 *N* sodium hydroxide. Determine the chloride in the neutral sample volumetrically with standard silver nitrate solution (approximately 0·1 *N*), using 2 drops of 0·1 per cent. dichlorofluorescein as indicator. This removes chloride and permits its determination. To precipitate cadmium hydroxide quantitatively, heat the solution to above 70° C, when the silver chloride coagulates, and add 2 ml of 0·05 per cent. thymolphthalein solution in 70 per cent. ethanol for every 100 ml of solution. Titrate with standard carbonate-free sodium hydroxide (approximately 0·1 *N*). Add the sodium hydroxide slowly, with constant stirring by gentle rotation. By careful approach, the end-point is reproducible to 0·02 ml. Evaluate the results, taking into consideration the appropriate blank end-point correction for the hydroxide titration (usually 0 to 0·2 ml).

The mole percentage of cadmium chloride in the binary salt mixture is given by—

$$\text{Mole percentage of cadmium chloride} = 100V_{Cd}/(2RV_{Cl}-V_{Cd}),$$

where V_{Cd} is the sodium hydroxide titre, V_{Cl} the silver nitrate titre and R is the ratio of the normality of the silver nitrate to that of the sodium hydroxide.

Results

Comparisons of the weight percentages of cadmium in the same salt mixtures by calculation from the known weights of cadmium chloride and alkali-metal chloride (synthesis) with those obtained electrolytically and by the above method are shown in Table I.

TABLE I

COMPARISON OF ELECTROLYTIC AND VOLUMETRIC METHODS FOR DETERMINATION OF CADMIUM

Cadmium in mixture taken, %	Cadmium found in mixture by electrolytic method, %	Cadmium found in mixture by volumetric method, %	Difference between cadmium taken and cadmium found by volumetric method, %
61·21	61·23	61·19	0·02
61·21	61·30	61·26	−0·05
57·41	—	57·36	0·05
48·63	—	48·61	0·02
43·52	43·50	43·58	−0·06

These results indicate that the volumetric method for determining cadmium as cadmium hydroxide can be as reliable as the electrolytic method and that the three consecutive titrations utilised do not interfere. No systematic error is evident. The volumetric method has the particular advantage in the work for which it was developed (and for cadmium chloride in general) of eliminating the weighing of comparatively small quantities of hygroscopic cadmium chloride. The method is also advantageous when rapid routine analyses are necessary.

REFERENCES

1. Bloom, H., and Richards, N. E., in preparation.
2. Bloom, H., and Barton, J. L., in preparation.
3. Sand, H. J. S., "Electrochemistry and Electrochemical Analysis," Blackie and Son Ltd., London and Glasgow, 1942.
4. Coetzee, J. F., *J. Chem. Soc.*, 1951, 997.
5. Britton, H. S., *Ann. Rep. Chem. Soc.*, 1943, 41.

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B.S. 245 : 1956. White Spirit. Price 6s.
B.S. 2690 : 1956. Methods of Testing Water Used in Industry. Price 17s. 6d.

Book Reviews

ADDENDUM 1955 TO THE BRITISH PHARMACOPOEIA 1953. Pp. xvi + 95. Published under the direction of the General Medical Council. London: The Pharmaceutical Press. 1955. Price 21s.

The Addendum 1955 to the British Pharmacopoeia 1953, official from the 1st of March, 1956, contains 56 monographs, covering 25 new drugs and 31 injections and tablets for drugs in the main text and in the Addendum, occupying the first 24 pages; these pages include monographs on a new Disintegration Test for tablets, Distilled Water and Purified Water.

In the revised Disintegration Test five tablets are submitted, simultaneously, to reciprocating motion under water in a tube with a gauze bottom, fitted with a "guided disk" for use with refractory samples; the test is conducted under specified conditions and in an apparatus of specified dimensions.

All the references to Distilled Water in the 1953 B.P. are now replaced by Purified Water, that is, water prepared either by distillation, or by treatment with ion-exchange material; except for use in injections, it may be supplied in place of Distilled Water when prescribed or demanded. The pyrogen test for Water for Injection has been revised.

Matters in which analysts will be more immediately interested are contained in the last 25 pages, in which there are two amendments to Appendix I of the Pharmacopoeia, Materials and Solutions Used, concerned with the weight per ml of *iso*Propyl Alcohol and the deposition of explosive compounds in Silver Ammonio-nitrate solution, and 29 additions to the reagent list.

* Obtainable from the British Standards Institution, Sales Department, 2 Park Street, London, W.1.

Five standard solutions have been added to Appendix II, and Titan Yellow, solution and paper, have been added to the list of indicators.

The arsenic limit for Centrimide is deleted and Ferrous Gluconate is added to Appendix VI, under the quantitative test for arsenic.

The table of quantities used in the Quantitative Test for Lead, Appendix VII, has suffered a deletion, an amendment and seven additions.

Alterations have been made to the alcohol limits for Tinctures of Lemon and Orange, and Syrup of Orange is deleted, in Appendix XII.

In Appendix XV alterations are notified under the headings: Standard Preparations to be Used in the Biological Assays and Tests Described in the British Pharmacopoeia, Antibiotics, Serological and Bacteriological Products, Hormones, Dextran Sulphate and the Test for Pyrogens.

In the section dealing with the assay of Vitamin A, the paragraph headed "Standard Preparation and Unit" has been revised and new matter on all-*trans* Vitamin-A Acetate has been added.

Additions and amendments have been made to Appendix XX under Dispensing of Parenteral Injections and the Test for Limit of Alkalinity of Glass.

The book is well produced, in conformity with the format and binding of the B.P.—perhaps too well produced for a book whose expectation of life is but two-and-a-half short years.

F. L. Okell

Hydrogen Ions: Their Determination and Importance in Pure and Industrial Chemistry. Volume I. By H. T. S. Britton, D.Sc., D.I.C., F.R.I.C. Fourth Edition. Pp. xix + 476. London: Chapman & Hall Ltd. 1955. Price 70s.

Professor Britton's book has been a familiar volume of reference for many chemists for just over 25 years, being first published in 1929. The second edition followed in 1932 and the third, in two volumes, in 1942; the last has been out of print for many years.

The new Volume I discusses the following topics: theory of electrometric methods for the determination of hydrogen-ion concentration, indicating and reference electrodes, the measurement of e.m.f., principles of volumetric analysis and standardisation of volumetric solutions, abnormal acids, theory of the ionisation of dibasic and polybasic acids, the activity theory of solutions, composition and standardisation of buffer solutions, the Lowry - Brønsted theory of acids and bases and, finally, colorimetric methods for the determination of pH.

Those familiar with the third edition will note that the lay-out of the book has not been changed, but the number of pages has been increased from 420 to 476. Most of the subject matter of that edition has been retained and extra paragraphs have been inserted wherever the author considered that more recent work was worthy of comment. A new chapter has been added to deal with the standardisation of buffer solutions; it includes a discussion of the extensive work carried out by the National Bureau of Standards in recent years and the decisions taken in 1950 by the National Bureau of Standards and by the British Standards Institution to assign a given pH value to one or more buffer solutions in preference to the use of a given electrode as the pH standard.

Much of the subject matter that has been retained is fundamental information that must always be readily available, but here and there the industrial chemist will consider that the text appears to be out of touch with present-day practice. Much of the equipment shown in the illustrations has an old-fashioned look, which is not in itself a bad thing, but the reader will look in vain for a discussion of direct-reading pH meters and the accuracy that might be expected of them. Again, the present-day analyst will be amused at the possibility of determining his titration end-point by means of a spectroscope (p. 446) and doubtless horrified that he might ever have to use the directions given on p. 455 for actually making his own indicators by organic synthesis, although this might be good for him occasionally.

Despite these quibbles, this is still the valuable reference book it always has been and its re-appearance will be generally welcomed.

A. G. Jones

Textbook of Pharmacognosy. By T. E. Wallis, D.Sc., F.R.I.C., F.P.S., M.I.Biol., F.L.S. Third Edition. Pp. xii + 578. London: J. & A. Churchill Ltd. 1955. Price 42s.

Originally published in 1946 as a successor to the "Textbook of Materia Medica" of the late Professor H. G. Greenish, Dr. Wallis's book was immediately accepted as the standard British work. In this edition the entire text has been scrutinised and, wherever necessary, revised in order to keep abreast of recent advances, while new commodities described herein for the first

time include Kaolin, Bentonite, Powdered Cocoa Shells, Fagopyrum (Buckwheat), Dubosia, Lucerne, Grass, Hamamelis, Rauwolfia, Visnaga, Pyrethrum Flowers and Belladonna Flowers.

Pharmacognosy may be defined as the scientific study of the structure of crude drugs of vegetable, animal and mineral origin, including their physical characteristics, cultivation and collection. It is with the macroscopical and microscopical structure that the analyst is most concerned, and this is the dominant theme of the book under review. The scope is conveniently indicated by quoting the titles to the chapters: Introduction; Starches; Powders of Natural Occurrence; Fossil Organisms, Shells and Minerals; Hairs and Fibres; Woods; Barks and Galls; Leaves; Flowers; Seeds; Fruits; Entire Organisms; Rhizomes and Roots; Unorganised Drugs; Gums and Saccharine Substances; Resins, Gum-Resins, Oleo-Resins; Fixed Oils, Fats and Waxes; Glands and Glandular Secretions; Commerce in Drugs. Anatomical structure is necessarily subsidiary to the discourse in the last six chapters, but is dominant in the others and the full and authoritative descriptions are supplemented with over 200 excellent drawings of both macroscopical and microscopical features.

In making an assessment of this distinguished work it should be realised that it was planned as a textbook for students of pharmacy, but the author has carried out his task so thoroughly that the book seems naturally to take its place as an important work of reference. For this reason it would be a valuable asset to a future edition to add further chapters dealing with the histology of culinary herbs, even though these are beyond the scope of the title. Detailed descriptions of many of the food flavouring spices are already included because they happen to be used pharmaceutically.

Each page reflects the erudition and patience of the author and he has been well supported by the publishers, who are to be congratulated upon having achieved a high standard of production at a modest cost.

N. L. Allport

Publications Received

A Textbook of Practical Organic Chemistry, including Qualitative Organic Analysis. By A. I. Vogel, D.Sc., D.I.C., F.R.I.C. Third Edition. Pp. xxviii + 1188. London, New York and Toronto: Longmans, Green & Co. Ltd. 1956. Price 60s.

Resonance in Organic Chemistry. By George Willard Wheland. Pp. xiv + 846. New York: John Wiley & Sons, Inc.; London: Chapman & Hall Ltd. 1955. Price 120s.

Laboratory Control of Dairy Plant. By J. G. Davis, D.Sc., Ph.D., F.R.I.C., M.I.Biol., F.R.S.H. Pp. xii + 395. London: Dairy Industries Ltd. 1956. Price 30s.

Quantitative Methods of Organic Microanalysis. By S. J. Clark, Ph.D., A.R.I.C. Pp. x + 253. London: Butterworths Scientific Publications. 1956. Price 30s.

Physical Chemistry. By Walter J. Moore. Second Edition. Pp. xii + 633. London, New York and Toronto: Longmans, Green & Co. Ltd. 1956. Price 30s.

The Determination of Toxic Substances in Air: A Manual of I.C.I. Practice. Edited by N. Strafford, C. R. N. Strouts and W. V. Stubbings. Pp. xxviii + 226. Cambridge: W. Heffer & Sons Ltd. 1956. Price 35s.

Papers for Publication in *The Analyst*

The Editor welcomes Papers and Notes for insertion in *The Analyst*, whether from members of the Society or non-members. They are submitted to the Publication Committee, who decide on their suitability for insertion or otherwise.

A copy of the current Notice to Authors, last published in full in *The Analyst*, 1956, **81**, 127, can be obtained on application to the Editor, *The Analyst*, 7–8 Idol Lane, London, E.C.3. All Papers submitted will be expected to conform to the recommendations there laid down and any that do not may be returned for amendment.

A few copies of the tabulated "Nomenclature of Vitamins," reprinted from *The Analyst*, 1953, **78**, 72, are also available.